The Effect of Glucose-Dependent Insulinotropic Polypeptide Infused at Physiological Concentrations on the Release of Insulin in Man

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Summary. Blood glucose and the responses of insulin and glucose-dependent insulinotropic polypeptide (GIP) were measured in 12 healthy, normal weight subjects after drinking 50 g glucose. On a subsequent occasion each subject was given a simultaneous infusion of GIP and glucose to mimic the plasma concentrations observed in the first test. The peak GIP concentration after oral glucose was 22.3 ± 1.9 pmol/l (mean $\pm$ SEM), but was higher after GIP infusion at 36.3 ± 4.6 pmol/l, ($p < 0.005$). The blood glucose levels following oral glucose peaked at 6.3 ± 0.5 mmol/l which was the same as seen after intravenous glucose. The insulin response to oral glucose was, however, far higher (431.3 ± 58.2 pmol/l) than that obtained after GIP and glucose infusion (191.6 ± 30.9 pmol/l, $p < 0.001$). Thus it has not proved possible to explain completely the oral enhancement of insulin release by the action of GIP alone.

Key words: Glucose-dependent insulinotropic polypeptide, gastric inhibitory polypeptide (GIP), insulin, blood glucose, enteroinsular axis, incretin.

Gastric inhibitory polypeptide, now commonly referred to as glucose-dependent insulinotropic polypeptide (GIP) is released by cells present in the upper small intestine in response to oral but not intravenous glucose [1]. It has been shown that GIP potentiates the release of insulin [2], but this is only demonstrable when glucose is elevated [3]. The evidence in support of this hormone playing a major part in the enteroinsular axis has been the subject of a recent review [4]. This study was designed to investigate the effect of

GIP on insulin release in the presence of physiological hyperglycaemia, as seen after a 50 g oral glucose load in healthy subjects.

Subjects and Methods

Subjects

The experiments were performed on 12 healthy volunteers (eight males and four females, mean weight 68.6 ± 3.2 kg, mean age 25.8 years, range 18–46 years). Informed consent was obtained from each subject, none of whom were taking any medication. Approval for the infusion of GIP was obtained from the Hammersmith Hospital Ethical Committee. Before investigation all subjects had been maintained on a normal unrestricted diet.

Test Protocols

Subjects were studied in a recumbent position after an overnight fast. Venous access was provided by 19 G indwelling catheters, separate arms being used for infusion and blood sampling.

Oral glucose: blood samples were taken before and for 120 min after ingestion of 50 g glucose, dissolved in 150 ml water.

Intravenous glucose and GIP infusion: both glucose and GIP were administered from syringes placed in separate, variable rate, constant speed syringe pump (Harvard Instruments, Massachusetts, USA). Glucose was given as 10% dextrose in 0.9% saline for 60 min. The rate of infusion was adjusted according to the capillary glucose concentration of samples taken from a warmed hand. The concentration of glucose was estimated by using glucose oxidase strips (Dextrostix, Ames, Bucks) and a portable photometer (Hypocount, Hypoguard, Suffolk). Information derived in this way was used to approximate the glucose curve obtained after oral glucose. The amount of glucose given varied according to the individual within a range of 10–18 g.

Pure porcine GIP (Professor J. C. Brown, Vancouver, Canada) was made up in 0.15 mol NaCl/l and 0.3 mmol human serum albumin/l (Lister Blood Products Laboratories, Elstree, Herts, UK) and administered as a 30 min infusion of 0.4 pmol $\text{kg}^{-1} \text{min}^{-1}$, followed by a 30 min infusion of 0.1 pmol $\text{kg}^{-1} \text{min}^{-1}$. This regimen had

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Fig. 1. Insulin response (pmol/l, mean $\pm$ SEM) in 12 subjects following 50 g oral glucose (●) or IV glucose + IV GIP (○). Solid bar denotes duration of infusion (see text for details)

been found, from a previous pilot experiment, to mimic approximately the plasma levels of GIP after a mixed evening meal. The infusion fluid was flushed through the system externally for 5 min to saturate the tubing. Samples of infusate were taken before and after each infusion dose to assess losses due to adsorption.

Glucose and Hormone Estimations

Blood glucose: 2.5 ml whole blood was taken into fluoride oxalate tubes which were kept refrigerated ($+4^{\circ}\text{C}$) until measurement by a glucose oxidase method for use with an auto-analyser.

Hormone estimations: 10 ml blood was taken into chilled lithium heparin tubes containing 1000 kallikrein inhibitory units (KIU)/ml aprotinin (Trasylol, Bayer, FRG). This blood was immediately centrifuged for 5 min at $1600 \times g$, the plasma decanted and stored deep frozen (-20°C) until measurement in a single assay (per hormone) to minimise the influence of any interassay error.

Insulin was measured using ^{125}I -labelled insulin (IM38: The Radiochemical Centre, Amersham, Bucks, UK) guinea-pig anti-insulin serum (Wellcome, Beckenham, Kent, UK) and human insulin standard (Wellcome, Beckenham, Kent, UK). Standard curve tubes were set up in human plasma cleared of endogenous insulin by charcoal adsorption. The incubation products were separated by dextran-coated charcoal. Sensitivity of the assay was 6 pmol/l.

Estimation of GIP was performed by radioimmunoassay using a mid C-terminal reacting antiserum (GIP 19) raised in a rabbit using carbodiimide condensation of pure porcine GIP to haemocyanin [5]. Radiolabelled antigen (^{125}I GIP) was prepared by lactoperoxidase oxidation [6]. The specific activity was approximately 65 Bq/fmol. In the absence of added unlabelled GIP, the antiserum used at a final dilution of 1:96,000 bound approximately 50% of 1.5 fmol of ^{125}I GIP added to each assay tube.

Standard curve plasma was prepared by the removal of endogenous GIP from human plasma by specific immuno-absorption [7]. Antiserum GIP 19 recognises two major immunoreactive forms of GIP. When analysed by gel permeation chromatography, the first peak elutes shortly after cytochrome C ($K_{av} 0.3 \pm 0.02$, mean of five estimations), the other peak co-eluting with pure porcine standard ($K_{av} 0.67 \pm 0.02$). The antibody demonstrated no cross-reactivity with any other gastro-intestinal or pancreatic hormones with the exception of glucagon, where it was less than 1%. The sensitivity of the assay allowed changes of 3 pmol/l GIP to be detected between individual adjacent plasma samples with 95% confidence.

Analysis of GIP Preparation for Infusion

To validate the molecular integrity of the GIP preparation used in this study the following tests were carried out:

Gel permeation chromatography: samples of the infusate diluted in plasma were loaded onto a $60 \times 0.9\text{ cm}$ Sephadex G50 superfine column (Pharmacia, Uppsala, Sweden) and eluted at 5 ml/h with 0.1 mol formic acid/l. Dextran blue, Cytochrome C and Na^{125}I were added to each run as internal markers of molecular size. Column calibration was carried out with 500 μl of human serum albumin to which 0.5 pmol pure porcine GIP and the internal markers were added at the beginning and end of the test. The column conditions remained stable throughout. All fractions collected were stored deep frozen and estimated in a single assay.

Disappearance half-time studies: six healthy volunteers received an infusion of $0.14\text{ pmol kg}^{-1}\text{ min}^{-1}$ of GIP for 60 min followed by an infusion of $0.39\text{ pmol kg}^{-1}\text{ min}^{-1}$ for a further 60 min. Blood samples were drawn from the opposite arm at 1, 3, 5, 7, 9, 11, 13, 16, 19, 21, 24, 27 and 30 min after plateau concentrations of $102.6 \pm 5.5\text{ pmol/l}$ had been reached.

Parallelism with pure porcine standard: four batches of the infusion fluid, taken after each dose, were diluted serially to give nominal concentrations of 4, 2.0, 1.0 and 0.5 pmol/ml and added to duplicate tubes interlocked with a standard curve under usual assay conditions.

Statistical Analyses

Results are expressed as mean and standard error of the mean ($\pm$ SEM, degrees of freedom = $n-1$). Where appropriate increments are denoted by Δ . Significance of results was assessed using Student's paired 't' test (two-tailed).

Results

The responses of insulin and GIP to both tests are shown in Figures 1 and 2 respectively. Following the oral glucose load, blood glucose rose from a mean basal of 4.1 ± 0.4 to $5.9 \pm 0.5\text{ mmol/l}$ (Δ glucose = 1.80 ± 0.6) at 30 min and reached a peak of 6.3 mmol/l (Δ glucose 2.2 ± 0.5) at 45 min. Plasma insulin rose briskly to $387.8 \pm 69.1\text{ pmol/l}$ at 30 min and reached a peak of $431.3 \pm 58.2\text{ pmol/l}$ at 45 min. Plasma GIP rose to a peak of $22.3 \pm 1.9\text{ pmol/l}$ at 30 min.

During the combined glucose and GIP infusion blood glucose rose to $6.3 \pm 0.3\text{ mmol/l}$ at 30 and

45 min from a mean basal of 4.3 ± 0.2 mmol/l (Δ glucose 2.0 ± 0.3) which was similar to that reached after oral glucose. The insulin response, however, was much reduced during this test with concentrations of 191.6 ± 30.9 at 30 min ($p < 0.005$) and 173.1 ± 27.9 at 45 min ($p < 0.001$). The plasma GIP obtained from infusion, however, increased from a mean basal of 9.1 ± 1.5 to 36.3 ± 4.6 pmol/l at 30 min ($p < 0.005$) and 26.3 ± 2.4 (NS versus oral glucose) at 45 min.

The GIP used for infusion eluted as a single peak after gel permeation chromatography. The elution coefficient (K_{av}) was 0.66 ± 0.04 which was the same as for human GIP from jejunal extracts, 5000 molecular weight GIP found in human plasma after oral glucose and porcine GIP from jejunal extracts; column recovery was $88 \pm 6\%$. The disappearance half time ($t_{1/2}$) of infused GIP in plasma was found to be 20.3 ± 1.1 min. The samples of infusate diluted in subject's plasma demonstrated full parallelism with the standard curve.

Discussion

Amongst the many gut factors proposed to exert incretin-like effects, the gut hormone GIP has become established as the main contender. In 1973 Dupre et al. [2] infused GIP to produce plasma levels of approximately 200 pmol/l and showed that this was potentially insulinotropic when blood glucose was raised by more than 60 mg/dl (3.2 mmol/l), giving a greater than twofold rise of insulin when compared with the response elicited by intravenous glucose alone. Andersen et al. [3] performed a series of experiments with a glucose clamp technique which demonstrated the glucose-dependent nature of GIP-stimulated insulin release. They gave oral glucose to release endogenous GIP and showed that no augmentation of the ensuing insulin release occurred during the maintenance of euglycaemia. However, when blood glucose was allowed to rise by 20 mg/dl (1.1 mmol/l) then the response of insulin could be enhanced. The same group reported in 1979 [8] that an infusion of GIP to give plasma levels of around 500 pmol/l produced a fourfold increase of insulin when blood glucose was clamped at an elevation of 54 mg/dl (3 mmol/l). The same high level of GIP produced a sevenfold increase of insulin when a hyperglycaemia of 143 mg/dl (8.0 mmol/l) over basal was maintained.

The results reported here are in broad agreement with those discussed above and show that, when blood glucose is raised by 2.0 mmol/l (36 mg/dl) above basal and GIP is infused to produce plasma concentrations within the physiological range, then a threefold rise over basal of insulin could be produced.

Fig. 2. GIP response (pmol/l, mean $\pm$ SEM) in 12 subjects following 50 g oral glucose (●) or IV glucose + IV GIP (○). Solid bar denotes duration of infusion (see text for details)

The hyperglycaemia produced in these subjects was comfortably in excess of the 20 mg/dl (1.1 mmol/l) increase of glucose proposed as the threshold for the insulinotropic action of GIP to become operative. However it is apparent from this investigation that the insulin response provoked by the infusion of GIP and glucose is only about one-third of that produced by the ingestion of the oral glucose load in spite of the similarity of the degree of hyperglycaemia observed. The insulinotropic effect of GIP (peak insulin 191.6 ± 30.9 pmol/l) plus glucose could well be greater than that seen after intravenous glucose alone when the results of this study are compared with those of eight healthy volunteers of similar age and weight (peak glucose 6.4 ± 0.3 mmol/l, Δ glucose 2.4 ± 0.2 , peak GIP 8.1 ± 2.1 pmol/l, Δ GIP 0.5 ± 1.8 , peak insulin 122.7 ± 20 pmol/l, Δ insulin 79 ± 13) (unpublished observations).

Without an agreed bioassay, it is conceivable that the porcine GIP used was not fully biologically active in man but indirect evidence from chromatography and parallelism studies suggest that the molecule is similar to that found in human jejunal tissue and circulating in the plasma. The disappearance half-time of the porcine GIP of 20.3 min was also similar to that found by Brown et al. [9] of 21 min and that found by Elahi et al. [8] of 20.4 ± 2.37 min. In addition, the administration of a low dose somatostatin infusion ($0.3 \text{ nmol kg}^{-1} \text{ h}^{-1}$) to six non-fasted patients with pancreatic apudoma, caused a suppression of endogenous GIP from a basal of 22.5 ± 3 pmol/l to 9.8

± 2 pmol/l after 30 min. When this data was plotted as a percentage of the GIP remaining against time, 50% had disappeared within 24 min (unpublished observation). Thus endogenous GIP was removed from the circulation with a similar time-course to the half-life of exogenous GIP. This further supports the similarity of the preparations used.

There are a number of differences in the experimental methods employed in this study and those reported earlier. Although the GIP infusion doses given by Elahi et al. [8] and the present authors were much the same, the plasma levels measured are disparate. We have consistently reported considerably lower fasting and post-prandial GIP concentrations [5, 10] and it is likely that differences in assay methodology, such as the use of standard curves bereft of GIP-free plasma, are responsible for this phenomenon. With regard to the potent insulin release reported in that paper, attention should be drawn to the size of the increment of blood glucose required to produce this (8.0 mmol/l) which would rarely be produced in subjects with normal glucose tolerance.

The use of procedures resulting in the endogenous release of GIP, reported by Andersen et al. [3], resulted in a slightly smaller enhancement of insulin when compared with the infusion experiment reported here, although the blood glucose levels in both studies were similarly elevated above basal. In this instance it is difficult to explore the effect of GIP on its own since clearly there may be potentiation by other gut factors, including neural influences, also stimulated by glucose ingestion and which may be insulinotropic in a glucose-dependent fashion.

We conclude from this study, and from the others [2, 3, 8], that the insulin response to oral glucose has not yet been shown to be due to the action of GIP alone. The effect of GIP appears likely to be weak in individuals with normal glucose tolerance where a minimal degree of hyperglycaemia can be observed. It may be that it is only in the event of supranormal blood glucose levels that a compensatory insulin stimulating mechanism involving GIP becomes important. The reportedly augmented GIP concentrations seen in some Type 2 (non-insulin-dependent) diabetics [11] may well be the result of some such compensatory action.

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