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Dissociated effects of glucose-dependent insulinotropic polypeptide vs glucagon-like peptide–1 on β-cell secretion and insulin clearance in mice

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Abstract

Glucagon-like peptide–1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) potently augment insulin response to glucose. It is less known what their effects are insulin clearance, which also contributes to peripheral hyperinsulinemia observed after administration of incretins together with glucose. The aims of this study were the quantification of C-peptide secretion and the evaluation of insulin clearance after administration of GIP with glucose. This allows the assessment of GIP's effects on hyperinsulinemia. In addition, GIP's effects were compared with those of GLP-1. Anesthetized female NMRI mice were injected intravenously with glucose alone (1 g/kg, n = 35) or glucose together with GIP (50 μg/kg, n = 12). Samples were taken through the following 50 minutes, and C-peptide and insulin concentrations were used to reconstruct C-peptide secretion rate and insulin clearance. In a previous study, GLP-1 (10 μg/kg) was used in 12 mice; and we used those GLP-1 results to compare GIP effects with those of GLP-1. C-peptide secretion rate peaked at 1 minute after glucose injection, and the immediate part of the insulin-releasing process was markedly augmented by both incretin hormones (1-minute suprabasal increment secretory rate was 20 ± 2 pmol/min for GIP and 28 ± 2 pmol/min for GLP-1, vs only 9 ± 1 pmol/min for glucose alone; $P < .001$). Until 10 minutes after administration, C-peptide secretion remained higher with incretins ($P < .0001$), whereas starting from 20 minutes, the 3 patterns were undistinguishable ($P > .2$). Insulin clearance, previously shown to be abridged by 46% with GLP-1, was reduced only by a nonsignificant ($P = .27$) 21% with GIP. This study thus shows that the 2 incretins markedly augment glucose-stimulated insulin secretion in mice by a preferential action on the immediate response to glucose of insulin secretion. However, the action of GIP is less effective than that of GLP-1. Insulin clearance with GIP is not significantly reduced. We conclude that GIP is less potent than GLP-1 in inducing glucose-stimulated hyperinsulinemia in the mouse.

1. Introduction

The incretin effect is the potentiation of insulin secretion after ingestion of glucose that occurs beyond and on top of the effect of glucose alone [1]. This has been nicely demonstrated in humans and mice by matching the glucose levels after intravenous and oral glucose [2,3]. The 2 most important incretin hormones are glucagon like peptide–1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) [4]. Glucagon like peptide–1 has also been of great interest because it has become a target for treatment of type 2 diabetes mellitus [5].

Both GIP and GLP-1 markedly potentiate glucose-stimulated insulin secretion in humans [6] and mice [7-9], leading to peripheral hyperinsulinemia. Nonetheless, the actual degree of augmenting insulin secretion at β-cell level by the 2 incretins in the mouse has not been established; in particular, it is not completely known whether GIP and GLP-1 exert, at maximal doses, similar effects in mice on β-cell insulin release, assessed with C-peptide kinetics.

Moreover, systemic insulin derives not only from the net effect of pancreatic insulin secretion but also from hepatic clearance. Interestingly, hepatic clearance of insulin may vary among species; but very little is known if incretins have a direct effect on this process. It is important to establish insulin clearance in the mouse because this

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animal model will most likely be used to test new generations of incretin-based therapeutic agents for type 2 diabetes mellitus. Recently, Meier et al [10] showed in humans that neither GLP-1 nor GIP seems to have a significant effect on reducing insulin clearance. In mice, instead, we have demonstrated that reduced hepatic insulin extraction contributes to the increased insulin levels observed after the administration of GLP-1 [11]. Whether GIP also affects insulin clearance in the mouse has not been yet established.

Therefore, the aims of the present study were (*a*) to directly quantify C-peptide secretion rate from the β-cell after an intravenous glucose bolus with and without concomitant administration of GIP; (*b*) to assess the clearance of insulin in the presence of GIP, for estimating the influence of insulin degradation rate on the peripheral hyperinsulinemia; and (*c*) to compare GIP's effects on insulin secretion and clearance with those of GLP-1, which have been previously quantified [11].

2. Materials and methods

2.1. Animals, experiments, and assays

Female NMRI mice, weighing 28.3 ± 0.9 g (range, 20.6-40.1 g), were purchased from Taconic (Skensved, Denmark). The animals were maintained in a temperature-controlled room (22°C) on a light-dark cycle of 12 hours each. Mice were fed a standard pellet diet (Lactamin, Stockholm, Sweden) and tap water ad libitum. The study was approved by the local animal ethics committee. Mice were anaesthetized after a 3-hour fast during the late morning hours with an intraperitoneal injection of midazolam (Dormicum; Hoffman-La Roche, Basel, Switzerland; 0.14 mg/mouse) and a combination of fluanisone (0.9 mg per mouse) and fentanyl (0.02 mg per mouse; Hypnorm; Janssen, Beerse, Belgium). After 30 minutes, a blood sample (75 μL) was taken from the retrobulbar, intraorbital, capillary plexus in a 100-μL pipette that had been prerinsed in heparin solution (100 U/mL in 0.9% NaCl; Lövens, Ballerud, Denmark). Either D-glucose alone (1 g/kg, n = 35; British Drug Houses, Poole, United Kingdom) or D-glucose together with GIP (50 μg/kg, n = 12; Peninsula Laboratories Europe, St Helens, United Kingdom) was injected in a tail vein. The procedure of the intravenous glucose test in mice and its use have been previously described in details [12,13]. Blood samples (75 μL each) were taken at 1, 5, 10, 20, 30, and 50 minutes; the total volume load was 10 μL/g body weight. Plasma was immediately separated and stored at −20°C until analysis. Insulin was determined by a double-antibody radioimmunoassay using guinea pig anti-rat insulin antibodies, ^{125}I-labeled human insulin, and, as standard, rat insulin (Linco Research, St Charles, MO). Mouse C-peptide was determined by a double-antibody radioimmunoassay using guinea pig anti-rat C-peptide antibody (cross-reacts 100% with mouse C-peptide), rat C-peptide standard, and ^{125}I rat C-peptide as tracer (Linco). Glucose was measured by the glucose oxidase technique.

Because GIP effects are to be compared with those of GLP-1, we recall the protocol already described previously [11], where synthetic GLP-1, at a dose of 10 μg/kg, was injected in 12 mice together with glucose. Previous studies have demonstrated that these doses of GLP-1 and GIP are those with the maximal effect in augmenting glucose-stimulated insulin secretion in mice [8,14].

2.2. Assessment of C−peptide and insulin dynamics

Insulin release (picomoles per minute) at the β-cell level is determined from C-peptide and insulin peripheral data by using a 2-compartment model of C-peptide kinetics, previously described in detail [11]. Briefly, the 2 pools represent intra- and extravascular space with irreversible loss occurring from the former; insulin kinetics is described with a single-compartment structure. With ad hoc kinetic experiments with exogenous bolus injection of biosynthetic C-peptide and insulin, we previously determined the fractional C-peptide clearance that was 8.2 ± 0.6% of volume per minute and the distribution volume of 8.4 mL in a 29-g mouse [11]. There was no significant effect of body weight on insulin and C-peptide kinetics. Insulin clearance after glucose injection alone and after glucose + GLP-1 experiments has been previously assessed in these animals [11]. We report again some of these findings in addition to new ones in the "Results" section for an easier comparison with those obtained in the present investigation with GIP.

2.3. Calculations and statistics

The deconvolution method, using the parameters reported above, provides the estimation of the unknown insulin secretion and posthepatic appearance profiles from the measured plasma concentrations during the intravenous glucose tolerance test for each mouse. The method also provides the total amount of hormone (picomoles) released during the considered time intervals. This method is commonly applied to C-peptide concentration measurements to obtain the prehepatic release because C-peptide has no first-pass elimination through the liver. In addition, combining C-peptide secretion and insulin peripheral appearance, the method also yields an estimation of insulin degradation [11]. Individual parameter estimates for each mouse were obtained by the nonlinear weighted least squares fitting based on the simultaneous analysis of all available data sets, that is the mixed-effects population modeling approach [15,16]. It accounts for intra- and interindividual variation in parameters and provides the protocol effect on C-peptide secretion rate and insulin liver degradation. An index of β-cell sensitivity to glucose was calculated as the ratio of the total amount of released C-peptide (picomoles) to the mean concentration (millimoles per liter) of glucose [17]. Data and results are reported as

means ± SE. Statistical comparisons between groups were performed by analysis of variance with Bonferroni post hoc analysis; $P < .05$ was considered significant.

3. Results

Basal glucose, insulin, and C-peptide were 9.6 ± 0.2 mmol/L, 302 ± 35 pmol/L, and 906 ± 55 pmol/L, respectively. The insulin to C-peptide molar ratio at baseline was 0.30 ± 0.03. C-peptide and insulin concentrations at 1, 5, and 10 minutes (Fig. 1) were significantly higher after glucose when combined with GIP than after glucose alone ($P = .00001$-$.02$). Glucagon-like peptide–1 results reported afterward derive from the study [11] and are used here for comparison with those of GIP. Comparing both results, it appears that at 5 minutes, C-peptide concentration with GIP was lower than that with GLP-1 ($P = .008$), whereas insulin was higher with GLP-1 both at 5 ($P < .02$) and 10 minutes ($P = .025$). Glucose pattern with GLP-1 was lower than that with glucose alone at every time point ($P = .00008$-$.0015$). With GIP, at 30 and 50 minutes, glucose was lower, though not significantly ($P = .07$).

Table 1 shows the average C-peptide (insulin) secretion in the various study groups. The total amount of suprabasal released hormone in the first 10-minute interval was 13.0 ± 1.8 pmol after glucose alone, 46.0 ± 3.1 pmol with glucose + GIP, and 77.2 ± 3.1 pmol with glucose + GLP-1. With both incretins, insulin secretion was significantly higher ($P < .0001$) than with glucose alone until 5 minutes. At time 5 minutes, the stimulation of insulin secretion by GIP was more than 2-folds lower than with GLP-1. In the interval 5 to 10 minutes, only mice with GIP and GLP-1 still had a significantly ($P < .0001$) elevated secretion, whereas with glucose alone, insulin secretion rate was not different ($P = .34$) from the preinjection rate. In the following intervals, no significant effect of the treatment on insulin secretion was found.

Both incretins highly augmented β-cell sensitivity to glucose: from 1.28 ± 0.06 $pmol_{CP}/(mmol/L)_{GLUC}$ with glucose alone to 5.57 ± 0.13 with GIP and 10.46 ± 0.16 with GLP-1 (both Ps $< .000001$), indicating that β-cell sensitivity was significantly lower with GIP than with GLP-1 ($P < .02$). As previously found [11], fractional insulin clearance when glucose was administered alone was 0.66 ± 0.11 min^{-1} and was 0.36 ± 0.10 min^{-1} with GLP-1, which is a 46% reduction when compared with glucose alone ($P = .04$). Fractional insulin clearance with GIP was 0.53 ± 0.10 min^{-1}, corresponding to 21% reduction with respect to glucose alone; but this reduction was not statistically significant ($P = .27$). A model-independent index of insulin clearance vs secretion was calculated as the ratio of the area under the concentration curves of insulin and C-peptide. These ratios were 0.312 ± 0.019 with glucose only and 0.331 ± 0.024 with GIP ($P = .54$), supporting the model-based results of an unchanged insulin elimination rate with GIP.

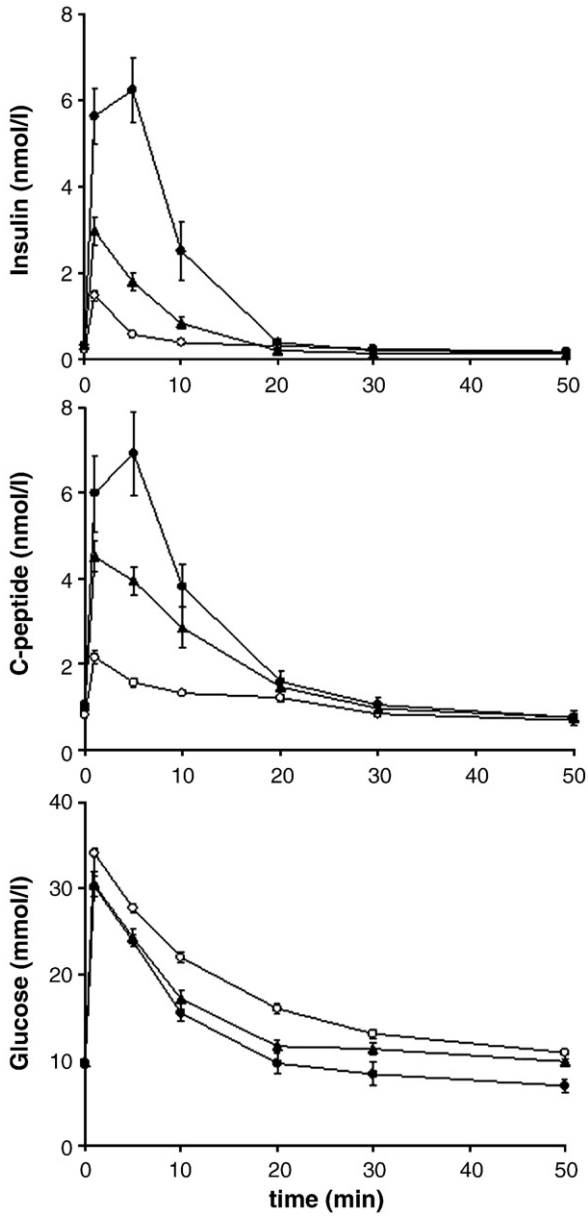

Fig. 1. Insulin, C-peptide, and glucose concentration (means ± SE) during intravenous glucose tolerance tests with glucose injection alone (1 g/kg, n = 35, open circles) and with additional administration of GIP (50 μg/kg, n = 12, closed triangles) or GLP-1 (10 μg/kg, n = 12, closed circles). Results with GLP-1 derive from a previously published study [11] and are reported here with permission for comparison with those of GIP.

4. Discussion

This study confirms the potentiation of GIP to augment glucose-stimulated insulin secretion. The novelty of the study is the demonstration that hyperinsulinemia observed after a concomitant administration of glucose and GIP in the mouse is almost exclusively due to increased β-cell secretion, rather than reduced hepatic insulin extraction as observed with GLP-1, which causes almost 50% reduction of insulin clearance [11]. Glucose-dependent insulinotropic

Table 1
Mean ± SE incremental C-peptide secretion rate (pmol/min) over basal release during different time intervals for the duration of intravenous glucose tolerance tests with glucose injection alone (1 g/kg, n = 35) and with additional administration of GIP (50 μg/kg, n = 12) or GLP-1 (10 μg/kg, n = 12)

Time intervals after glucose bolus (min)	Glucose only	Glucose + GIP	Glucose + GLP 1
0-1	8.60 ± 1.03	20.37 ± 1.75	28.33 ± 1.76
1-5	0.91 ± 0.31	4.23 ± 0.53	9.75 ± 0.50
5-10	0.16 ± 0.16	1.74 ± 0.27	1.97 ± 0.30
10-20	−0.12 ± 0.05		
20-30	−0.21 ± 0.04		
30-50	−0.09 ± 0.03		

The incremental secretion curves are indistinguishable from time 10 to 50: the mean of all experiments is shown. Significant differences ($P < .01$) between glucose + GIP and glucose + GLP-1 only from 0 to 5 minutes. Part of the results with GLP-1 were already described in figure 4 of Ahrén et al [11]; here they have been repeated for easier comparison with those obtained with GIP.

polypeptide instead only induces a nonsignificant 21% reduction. This is the first study that examines the effects of the 2 incretin hormones on insulin clearance in the mouse and compares their relative effects. In this regard, it is necessary to point out that GLP-1 results derive from a previously published study [11] in animals of the same strain and coming from the same lot of the mice supplier. Here, those results are reported to facilitate the comparison of GIP effects with those of GLP-1.

Recently, Meier et al [10] showed in humans that the reduction of hepatic insulin clearance is mediated by neither GLP-1 nor GIP. However, this study was carried out by evaluating only the molar ratio of insulin to C-peptide, which is known to be valid only in steady-state (fasting) conditions and not during dynamic experiments because of the different kinetics of the 2 peptides [18]. Our study exploits a previously developed model of C-peptide and insulin kinetics in mice, which yields the assessment of insulin clearance in dynamic conditions, and shows that, at variance with GLP-1, GIP has no significant effect on hepatic insulin clearance even in dynamic conditions. The virtually identical insulin to C-peptide molar ratio before the glucose injection with and without incretins shows that the possible changes in insulin degradation occur only during the dynamic part of the test, that is, after incretins are given; therefore, incretins can be considered responsible for those changes. To the best of our knowledge, this is the first time that GIP and GLP-1 are compared in terms of their effect on insulin clearance.

To quantify the contribution of β-cell secretion to peripheral hyperinsulinemia, without the confounding effects of insulin hepatic extraction, we have analyzed C-peptide, which is secreted equimolarly with insulin, but not degraded in the liver. In fact, analyzing only insulin concentration data could be in certain circumstances misleading because they may hide also the contribution of the clearance rate, which we have shown may vary with changing incretins [11]. Insulin clearance is important from a clinical point of view because the aim of any insulin-based therapy is to reach and maintain specific insulin levels in periphery. This is achieved either by directly administering insulin or by giving secretagogues; and knowing the clearance rate of insulin is important because changes in this process will affect insulinemia, for instance, the higher the clearance is, the higher must be the dose to maintain the desired peripheral concentration. In regard to incretins, it is therefore of interest to examine whether the incretin-induced marked increase of insulinemia is due not only to increased β-cell sensitivity to glucose, but also to reduced insulin clearance.

The arterovenous differences through the liver together with assessment of blood flow and portal measurements would provide a direct evaluation of hepatic extraction. Because this technique was not used in this study, we relied on modeling techniques, which have been proven to be able to estimate β-cell secretion and liver degradation [17]. Here, we used deconvolution that allows the reconstruction of the profile of secretion rates at the portal level from peripheral concentration data in a single individual or animal [18]. We observed a marked potentiation of the immediate response to glucose of C-peptide secretion rate. This immediate response was equipotently augmented by both incretins, given at the doses known to elicit the maximum effect [8,12,14]. In contrast, at subsequent intervals, GIP was less potent, suggesting a less prolonged action, as evident in the interval 1 to 5 minutes. The effects of that dose of GIP completely faded away between 10 and 20 minutes after its administration with glucose. The same happened for GLP-1. The index of β-cell sensitivity to glucose provides an additional good indicator of the incretin effect on glucose-stimulated β-cell release. It showed a markedly significant increase of the sensitivity to glucose in the presence of both incretins. Yet, this sensitization was much less with GIP than with GLP-1.

The receptors for GIP and GLP-1 are both G-protein–coupled receptors that signal through an isoform of G-proteins ($G\alpha_s$) that couple to an activation of adenylate cyclase with increased concentration of cyclic adenosine monophosphate [19]. Furthermore, both incretins show similar metabolic fates in that they undergo rapid inactivation in the circulation by the enzyme dipeptidyl peptidase–4 [20]. Nonetheless, the less protracted action of GIP vs GLP-1 would suggest either a quicker inactivation of GIP or a more prolonged activation of the GLP-1 receptor than that of GIP. This is probably achieved by different binding characteristics.

Incretin hormones also affect glucagon secretion; thus, their role is fundamental for the overall effect on glucose homeostasis [21] because the inhibition of glucagon secretion contributes to the improvement of glycemia after GLP-1–based therapy [22]. In the present study, however, we did not determine glucagon because our results are based on the kinetics after glucose administration, which is a condition when glucagon secretion is markedly suppressed. Nevertheless, the potential influence of glucagon on insulin clearance would be worth further studies.

In summary, our results show dissociated effects of the 2 incretins in mice. In particular, hyperinsulinemia after concomitant administration of GIP and glucose is mostly due to an elevated β-cell secretion. With the same doses of GLP-1 and glucose, peripheral hyperinsulinemia is more prominent; and it is due to concomitant effects of a higher insulin secretion and a markedly reduced degradation. In general, the β-cell is less sensitive to glucose in the presence of GIP than of GLP-1. Because therapies based on the incretin effect are currently used in the treatment of type 2 diabetes mellitus, it is of interest that our observational study has demonstrated directly that GLP-1, besides reducing the clearance of the released insulin, so markedly augments the β-cell sensitivity to glucose, resulting in a striking prominent immediate insulin response. It is in fact well known that the early releasing phase is most readily impaired during development of type 2 diabetes mellitus [23] and that is considered a target for pharmacologic intervention. On the other hand, GIP appears to be less potent and thus probably less appealing as a pharmacologic agent.

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