Immunoblockade of Endogenous Glucagon-Like Peptide-1 by Monoclonal Antibodies in Conscious Rats: Effect on the Insulin Response to Intragastric Glucose

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The physiological action of endogenous active forms of glucagon-like peptide-1 (GLP-1) on the insulin response to intragastric glucose was studied in conscious male Wistar rats by immunoblockade with two monoclonal antibodies directed against different epitopes of GLP-1(7-36)amide. Plasma concentrations of intraperitoneally injected monoclonal antibodies were determined before and during each experiment by an enzyme-linked immunosorbent assay (ELISA) specific for GLP-1–binding antibodies. Three hours after injection of the two monoclonal antibodies, the plasma insulin response (area under the curve) following intragastric glucose 1 g/kg was reduced to a mean level (mean ± SEM) of 60% ± 8% (n = 11) of control responses previously determined in the same rats, and the time course of the response showed almost no increase in insulin during the first 10 minutes, reaching a maximum of 45.1 ± 4.6 μU/mL at 30 minutes, in contrast to the rapid increase of the control response to a maximum of 64.5 ± 5.1 μU/mL at 15 minutes. Total C-terminally amidated GLP-1 measured by radioimmunoassay (RIA) of acid ethanol–extracted plasma increased from a mean basal level of 10 ± 2 pmol/L to a peak of 31 ± 5 pmol/L at 15 minutes in the control experiments, while basal and response levels greater than 100 pmol/L were recorded after antibody treatment. The increase of plasma glucose was reduced in the presence of the antibodies, peaking at a mean of 9.7 ± 0.3 mmol/L at 30 minutes, compared with 11.8 ± 0.5 mmol/L at 30 minutes in the control experiments. The action of GLP-1 appears particularly important for the early insulin response to ingested glucose, and the unexpected effect of the antibodies on the glucose response may point to a net promoting effect of GLP-1 on intestinal glucose absorption.

THE BIOLOGICALLY ACTIVE forms of glucagon-like peptide-1 (GLP-1), GLP-1(7-36)amide and GLP-1(7-37), secreted by intestinal endocrine cells in response to nutrient,[1,2] nervous,[3,4] and hormonal[4,5] stimuli, are potent hormones that stimulate insulin secretion in the presence of glucose.[6-8] As such, they are believed to form a significant component of the entero-insular axis, by which insulin secretion is regulated in accordance with nutrients arriving in the small intestine. However, measurement of the physiological levels of these peptides in plasma has not been without problems, since any antibody not specifically directed against the free termini of the active peptides will also recognize less active or inactive extended forms that are present in the circulation. This has been an obstacle for straightforward physiological studies of GLP-1 action, and most studies have been of a pharmacological nature in which the relevant synthetic peptide has been injected or infused to produce concentrations that in most cases are supraphysiological to a greater or lesser degree.

A notable advance was obtained when it was discovered that exendin(9-39)amide from Gila monster venom was a potent antagonist of the GLP-1 receptor.[9] This has enabled determination of the effects of GLP-1 receptor blockade on insulin secretion and other parameters.[9,10] However, such experiments are open to the objection that exendin(9-39)amide may have effects beyond blocking the exendin 4/GLP-1 receptor, and that GLP-1 may also exert various extrapancreatic effects[11,12] that may not all be mediated by this receptor (a view that remains controversial).

Another way of studying the physiological role of the active forms of GLP-1 is to block their effects by means of specific antibodies against these forms. If the antibodies are directed against epitopes known to be involved in the physiological actions of the peptides, as determined by pharmacological studies on synthetic peptide analogs,[13,14] and are used at sufficient levels to reduce the free active peptide concentration to the subphysiological range, this can provide a direct estimation of the quantitative role of these peptides in, eg, the insulin response to ingested nutrients. The validity of such experiments is also, of course, subject to the specificity of the antibodies to the peptides concerned and to the absence of any independent effects of the antibodies themselves. Such experiments, in eliminating hormonal action without directly attacking the hormone receptors involved, will provide a useful complement to the results obtained by receptor blockade with exendin(9-39)amide.

We have developed a series of mouse monoclonal antibodies against GLP-1(7-36)amide, some directed against an epitope that includes the C-terminal amide group, and others against more N-terminally situated epitopes that do not, however, include the free N-terminus of the peptide. The affinity constants of the individual antibodies are in the order of 10^7 L/mol. In the present study, we have used a combination of two of these antibodies, one C-terminal amide-specific and one capable of binding to the peptide in the presence of the other, to obtain a higher effective avidity and thus to block, presumably by a mechanism of steric hindrance, access of the peptide to its receptors at antibody concentrations that can be obtained in rats in vivo. The effect of pretreating conscious rats with these antibodies on the insulin response to intragastric glucose has

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been determined, and shows not only a quantitative diminution of the insulin response but also a notable change in the time course, whereby the early part of the response is profoundly suppressed.

MATERIALS AND METHODS

Mouse Monoclonal Antibodies

Mouse monoclonal antibodies 147-6 and 147-7 were obtained from *Bacillus Calmette-Guérin* (BCG)-preimmunized mice injected intraperitoneally with GLP-1(7-36)amide linked to purified protein derivative (PPD) of tuberculin with glutaraldehyde. The antibodies were purified from concentrated hybridoma supernatant on 1-mL Econo-Pac protein A cartridges (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions and dialyzed against phosphate-buffered saline ([PBS] 10 mmol/L sodium phosphate buffer, pH 7.4, containing 130 mmol/L NaCl). Protein concentrations were determined from the absorbance at 280 nm, assuming a value of 1.35 for a 1-mg/mL solution of immunoglobulin G. Affinity constants of these antibodies for GLP-1(7-36)amide were determined by inhibition enzyme-linked immunosorbent assay (ELISA) as 1.5×10^7 L/mol for 147-6 and 1.6×10^7 L/mol for 147-7. Specificity determination by direct and indirect ELISA procedures showed that antibody 147-6 was directed against an epitope that included the amidated C-terminus of GLP-1(7-36)amide, whereas antibody 147-7 reacted with a nonoverlapping epitope situated N-terminally to the 147-6 epitope. Antibody 147-6 cross-reacts fully with GLP-1(1-36)amide but shows less than 0.2% cross-reactivity with GLP-1(7-37), human GLP-2 (hGLP-2), glucagon, and human gastric inhibitory peptide (hGIP) in inhibition ELISA. Antibody 147-7 cross-reacts fully with GLP-1(1-36)amide and GLP-1(7-37), but shows less than 0.2% cross-reactivity with hGLP-2, glucagon, and hGIP.

The capacity of antibodies 147-6 and 147-7 to inhibit GLP-1–stimulated insulin secretion was verified in the isolated perfused rat pancreas preparation.[8] At a concentration of 20 μg/mL in perfusion solution containing 6.6 mmol/L glucose, each of these antibodies completely inhibited the insulin secretory response to 1 nmol/L GLP-1(7-36)amide observed in control perfusions without antibody (O. García Hermida, T. Fontela, and present authors, unpublished data, 1995).

RIA

The immunoreactive insulin level was measured by an Immuchem insulin RIA kit for human insulin assay (ICN Pharmaceuticals, Costa Mesa, CA) in accordance with the manufacturer's instructions using the supplied human insulin standards, label, and quality controls. The antibody used was stated by the manufacturer to cross-react 95% with rat insulins; assay results were therefore used directly, without correction for the small difference in reactivity between rat insulins and human insulin.

RIA for the amidated forms of GLP-1 (GLP-1(7-36)amide and GLP-1(1-36)amide) in rat plasma was performed as previously described,[15] with plasma samples extracted in HCl-ethanol to remove substances interfering with antibody binding of the peptides. The validity of the extraction method and RIA for determining amidated GLP-1 concentrations in rat plasma containing high concentrations of the monoclonal antibodies against GLP-1(7-36)amide was controlled by extracting and assaying a standard curve of GLP-1(7-36)amide incubated with peptide-free (charcoal-adsorbed) rat plasma[15] containing the monoclonal antibodies at a combined concentration of 160 μg/mL. This resulted in recovery rates of 62% to 100% (mean, 77%) of the GLP-1(7-36)amide added over the range of 2 to 10 fmol/100 μL plasma.

ELISA

The ELISA of functional monoclonal antibody, ie, antibody still capable of binding GLP-1(7-36)amide, was performed in 96-well polystyrene microwell plates (MaxiSorp; Nunc, Roskilde, Denmark) coated overnight at 4°C with GLP-1(7-36)amide 1 μg/mL in 0.05 mol/L sodium carbonate, pH 9.6, 100 μL/well. Plates were washed between each step with three changes of washing buffer consisting of PBS made 0.5 mol/L in NaCl and containing 0.1% vol/vol Tween 20. Subsequent layers were applied in dilution buffer consisting of washing buffer to which 1% wt/vol bovine albumin was added. Plasma containing monoclonal antibody against GLP-1(7-36)amide was diluted 1:100, added to the first column of wells at 200 μL/well, and then serially double-diluted across the plate in 100-μL volumes. After 1 hour of incubation with shaking at room temperature, the plate was washed and incubated for another hour with horseradish peroxidase–conjugated rabbit antibody against mouse Ig (P260; Dako, Glostrup, Denmark) at a dilution of 1:1,000. After washing, 100 μL substrate solution containing *o*-phenylenediamine 0.04% wt/vol and hydrogen peroxide 0.014% wt/vol in 0.1 mol/L citrate-phosphate buffer, pH 5, was added to each well. The colorigenic reaction was allowed to proceed for 30 minutes in the dark at room temperature, and was stopped by adding 150 μL 1-mol/L sulfuric acid per well. The optical density of solutions in the wells was read at 492 nm in an EAR 400 FT plate reader (SLT-Labinstruments, Groedig, Austria), subtracting background at 620 nm.

Mono-[^{125}I]-GLP-1(7-36)Amide Binding by Rat Plasma Samples

Remnants of plasma samples from time points 0 to 60 minutes in control experiments and 3 hours after antibody injection were respectively pooled for individual rats. The ability to bind GLP-1 was assessed by adding mono-[^{125}I]-GLP-1(7-36)amide, prepared as previously described,[15] at 4.5 fmol/100 μL plasma and incubating overnight at 4°C. Bound and free peptide were then separated with charcoal as for the RIA.[15] The amount of labeled peptide added was determined by self-displacement on the RIA standard curve.

Plasma Glucose Determination

Plasma glucose concentrations were determined on 10-μL aliquots of plasma by the glucose oxidase method using a commercial kit (Glucose GOD-Perid; Boehringer, Mannheim, Germany).

Experimental Design

Male Wistar rats (187 to 215 g body weight) maintained under controlled conditions of temperature and illumination with free access to water and standard food pellets were trained to tolerate immobilization in a restraining cage, intraperitoneal injection of sterile saline, blood sampling from the tail tip, and intragastric gavage of tap water. Toleration training was performed on each of 3 days preceding the control experiment, and consisted of subjecting the rats to a simulated experimental protocol in which only minimal amounts of blood were taken.

In a preliminary experiment, the time course for the appearance of mouse monoclonal antibody in plasma after intraperitoneal injection of 1 mg antibody in a 200-g rat was determined. Blood samples were taken from the tail at 0 minutes, 30 minutes, and 1, 2, 3, and 4 hours after injection; the plasma was separated and analyzed for functional antibody by ELISA as already described. Control normal rat plasma to which monoclonal antibody was added directly at a concentration of 0.1 mg/mL was included to enable the absolute values for antibody concentration to be calculated. A maximal plasma antibody concentration of 56 μg/mL was reached 3 hours after intraperitoneal injection.

The control experiment with intraperitoneal injection of vehicle alone was performed first in each rat to avoid interference from the persistent effects of prior antibody treatment. One week later, the protocol was repeated with intraperitoneal injection of the two monoclonal antibodies. The general protocol for control and antibody experiments was as follows. Rats were deprived of access to food overnight but had free

access to water. At 10 AM, they were injected intraperitoneally either with vehicle alone or with 5 mg/kg antibody 147-6 plus 5 mg/kg antibody 147-7 dissolved in 1 mL PBS. After 3 hours, the rats were placed in a restraining cage, a basal blood sample was taken from the tail, and 1 g/kg glucose was administered intragastrically as a 25% wt/vol solution in distilled water. Blood samples (0.5 mL) were collected from the tail at 5, 10, 15, 30, and 60 minutes after glucose, into ice-cold Eppendorf tubes containing buffered EDTA (0.6 mg) and aprotinin (100 kallikrein inhibitory units) dried onto the tubes. Plasma was separated immediately and stored in aliquots at −20°C for determination of immunoreactive insulin and glucose, while a further aliquot was extracted in HCl-ethanol as described[15] for RIA of C-terminally amidated forms of GLP-1. Basal plasma samples were also analyzed for functional monoclonal antibody by ELISA as already described.

Data Analysis

Results are presented as the mean ± SEM. The size of the plasma insulin and glucose responses (incremental area under the curve) was estimated by calculating the area under straight lines joining the plasma concentrations at successive time points and subtracting the area under a horizontal straight line drawn through the lowest concentration, whenever it occurred. If the responses were initially negative, only the positive responses following the time point of lowest concentration were included. Differences between control and experimental responses were analyzed by Wilcoxon's test for paired data. Data were also analyzed by Kendall's rank correlation coefficient (τ).

RESULTS

Plasma Concentration of Monoclonal Antibodies

The total plasma concentration of monoclonal antibodies still able to bind GLP-1(7-36)amide 3 hours after intraperitoneal injection was estimated by ELISA of basal plasma samples obtained immediately before intragastric glucose gavage. The values were 101 to 251 μg/mL, with a mean of 161 ± 17 μg/mL.

Plasma Binding of Mono-[^{125}I]-GLP-1(7-36)Amide

Plasma samples taken during the control experiments were unable to bind radioactively labeled GLP-1(7-36)amide, producing values equivalent to blank binding in RIA buffer. Plasma taken after injection of the monoclonal antibodies showed binding of the added label (corrected for blank) between 66.9% and 80.6% (mean, 76.9% ± 1.2%) for individual rats. The percentage of bound label was strongly correlated with the measured antibody concentration ($\tau = .81, P < .001$).

Plasma Insulin Response

The plasma insulin response to intragastric glucose was notably delayed and decreased by prior injection of the two monoclonal antibodies (Fig 1). The mean basal plasma insulin concentration was not significantly different 3 hours after control or antibody injection (25.5 ± 2.9 and 24.4 ± 2.8 μU/mL, respectively). Whereas in the control experiments, the mean plasma insulin increased rapidly during the first 10 minutes to reach a peak of 64.5 ± 5.1 μU/mL at 15 minutes, after injection of the antibodies, there was little increase in plasma insulin during the first 10 minutes, whereafter it increased to reach a lower peak of 45.1 ± 4.6 μU/mL at 30 minutes. In terms of the area under the curve, the positive responses were reduced from a mean of 1.630 ± 0.197 mU · min · mL^{-1} in control experiments to 0.985 ± 0.133 mU · min · mL^{-1} in the presence of the antibodies, representing 60% ± 8% of the mean control response. Despite the marked reduction of the insulin response in most of the rats, three rats with below-average control insulin responses showed some increase in the area under the curve in the presence of the antibodies (Fig 2). This resulted in statistical significance ($P < .05$) by Wilcoxon's test for paired values. No significant correlation was found between the magnitude of the effect on the insulin response and plasma concentrations of functional monoclonal antibody over the range observed ($\tau = -.15, P > .5$).

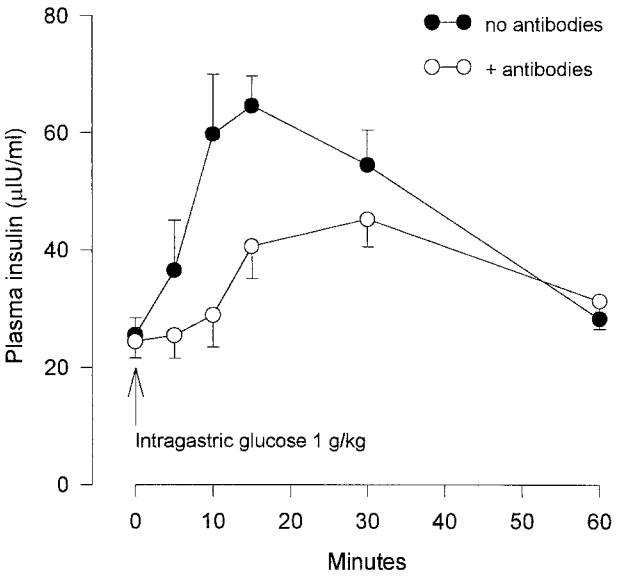

Fig 1. Time course of the mean plasma insulin response (error bars show SEM) to intragastric glucose in 11 rats under control conditions (●) and 3 hours after intraperitoneal injection of 2 monoclonal antibodies against GLP-1(7-36)amide (○). Antibodies 147-6 and 147-7 were injected at a dose of 5 mg/kg each, producing plasma functional antibody concentrations of 101-251 μg/mL at the time of glucose administration. Plasma insulin was measured by RIA.

Plasma Glucose Concentration

The mean basal plasma glucose concentration was identical after control or antibody injection (4.9 ± 0.2 mmol/L in both cases). Although plasma glucose peaked at 30 minutes in both control and antibody experiments, the mean rate of increase and the peak concentration achieved was significantly lower in the presence of the antibodies (Fig 3). The mean glucose concentration reached 11.8 ± 0.5 mmol/L at 30 minutes in control experiments, whereas the corresponding value in the presence of antibodies was 9.7 ± 0.3 mmol/L. This was reflected in the values for the area under the curve (Fig 2), which declined from a mean of 324 ± 23 mmol · min · L^{-1} in controls to 220 ± 15 mmol · min · L^{-1} in the presence of antibodies ($P < .01$ by Wilcoxon's test for paired values). There was no significant correlation between the magnitude of the effect on the glucose increase and plasma concentrations of functional monoclonal antibody over the range observed ($\tau = -.33, P > .1$).

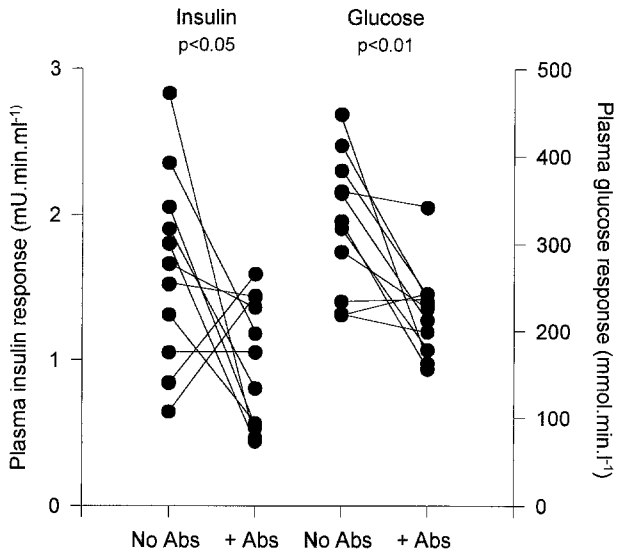

Fig 2. Plasma insulin and glucose response to intragastric glucose 1 g/kg in 11 individual rats under control conditions (No Abs) and 3 hours after intraperitoneal injection of 2 monoclonal antibodies against GLP-1(7-36)amide (+Abs). Responses were calculated as the incremental area under the curve of the positive response following the lowest plasma concentration recorded. The significance of differences in response was calculated by Wilcoxon's test for paired data.

Plasma Response of C-Terminally Amidated GLP-1

In the control experiments, total C-terminally amidated forms of GLP-1 increased from a mean basal plasma concentration of 10 ± 2 pmol/L to a peak at 10 minutes (30 ± 5 pmol/L) or 15 minutes (31 ± 5 pmol/L) after intragastric glucose gavage (Fig 4). However, in the presence of monoclonal antibodies, both basal and subsequent plasma concentrations were measured as more than 100 pmol/L. Control extraction and assay of GLP-1(7-36)amide incubated with rat plasma containing 160 μg/mL of the same monoclonal antibodies showed that this result was not an extraction or assay artifact due to the antibodies.

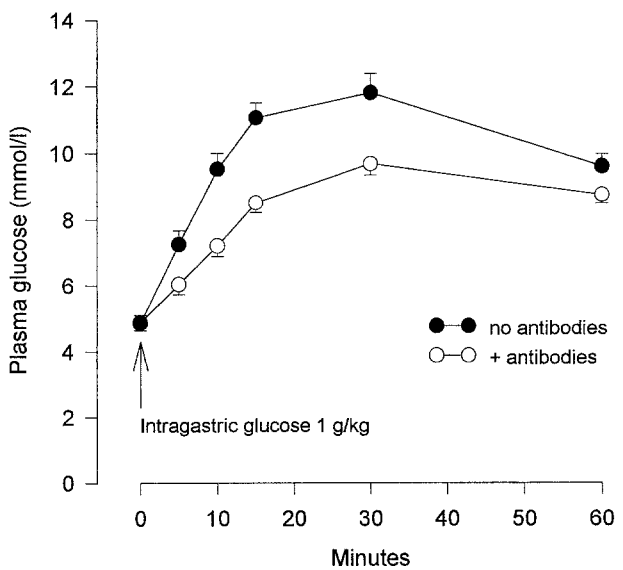

Fig 3. Time course of the mean plasma glucose response (error bars show SEM) to intragastric glucose in 11 rats under control conditions (●) and 3 hours after intraperitoneal injection of 2 monoclonal antibodies against GLP-1(7-36)amide (○). Plasma glucose was measured by a glucose oxidase method.

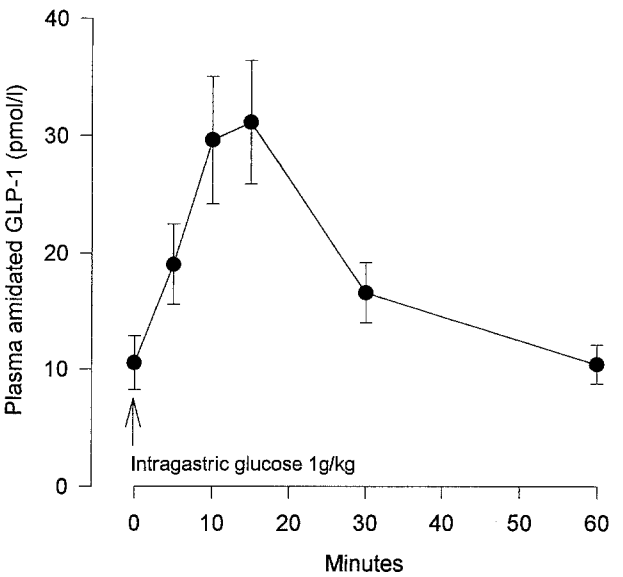

Fig 4. Time course of the mean plasma response of C-terminally amidated forms of GLP-1 (error bars show SEM) to intragastric glucose in 11 rats under control conditions. All values obtained after intraperitoneal injection of the 2 monoclonal antibodies against GLP-1(7-36)amide were off-range at >100 pmol/L. Total C-terminally amidated GLP-1 was measured by RIA of plasma samples extracted with HCl-ethanol, which removed plasma interference and was shown in control assays to quantitatively liberate GLP-1(7-36)amide bound to monoclonal antibodies.

DISCUSSION

The experimental protocol was designed to permit the use of conscious rats without intravenous infusion, and to allow paired comparison of control and experimental results in the same animals. Because of possible long-term effects of antibody treatment and the uncertain antibody washout time, we considered it inadvisable to randomize the order of antibody and control experiments, opting to perform the control experiment first in each case, followed 1 week later by the antibody experiment. This introduces a time variable, which we nonetheless judged as likely to be of little importance in the adult rats used, in comparison to the major advantage of paired comparisons and avoidance of effects due to inadequate antibody washout.

Comparison of the time course and magnitude of the mean insulin response to glucose in the presence and absence of antibodies to GLP-1(7-36)amide indicates that the active forms of GLP-1 significantly stimulate this response, with the effect particularly notable during the first 10 to 15 minutes after glucose reaches the stomach. This contrasts with the predominantly distal location of all forms of GLP-1, and indeed all proglucagon processing products, in the small intestine.[16-19] However, an increase in the plasma level of amidated forms of GLP-1 is already apparent at 5 minutes, and our results suggest that the mean increase of about 8 pmol/L that occurs during this time in control experiments is sufficient to contribute signifi-

cantly to the early stimulation of insulin release in the presence of plasma levels of glucose that have increased by 1 to 2 mmol/L during the same time. The speed of the GLP-1 response despite the distal location of the peptide in the intestine points to the importance of rapidly acting nervous and humoral stimuli to GLP-1 release from the intestine. Intestinal GLP-1 secretion is potently stimulated by cholinergic agonists acting on atropinic receptors,[3,4,20] and is thus under the presumptive control of parasympathetic autonomic reflexes affecting the small intestine. In addition, bombesin is a powerful stimulant of intestinal secretion of proglucagon processing products[4,21,22] and peptides of the bombesin family strongly stimulate GLP-1 release,[5,23,24] providing a neuropeptide-mediated mechanism that may supplement the stimulus due to the classic neurotransmitter acetylcholine. A further mechanism is the powerful stimulatory endocrine effect of GIP on GLP-1 release.[3,5,23] In contrast to GLP-1, GIP is predominantly located in the duodenum,[25] and is thus strategically placed to respond to glucose derived from carbohydrates leaving the stomach. It is postulated that GLP-1 is released from the distal intestine in response to elevated plasma levels of GIP rather than to any arrival of glucose in this region.[5,23]

We attribute the inhibition of GLP-1 effects by the two monoclonal antibodies to prevention of the interaction of active forms of the peptide with their receptor by a steric effect of the attached antibodies. The specificity of this action depends on the specificity of attachment of the antibodies to the active peptides and their lack of interaction with other components of the stimulatory system, be they components of the nervous system and their corresponding receptors, GIP and its receptors, or GLP-1 receptors. This is not an exhaustive list, because there is also the theoretical possibility of unpredictable pharmacological effects, unrelated to GLP-1 binding, of high concentrations of the antibodies on other systems. Antibody 147-6 is specific for the amidated C-terminal of GLP-1(7-36)amide or GLP-1(1-36)amide and does not bind to GLP-1(7-37), the minority active form, or to GLP-2, glucagon, or GIP. Antibody 147-7, on the other hand, is directed against a more N-terminally situated epitope, but as it reacts with both active and N-terminally extended forms of GLP-1, it must be presumed to be a "side-reading" antibody. It reacts fully with GLP-1(7-37), but shows no cross-reaction with GLP-2, glucagon, or GIP. Both antibodies can be attached simultaneously to GLP-1(7-36)amide, as shown by their respective use as capture and detecting antibodies in ELISA for GLP-1. However, only antibody 147-7 will attach to GLP-1(7-37). Whether the attachment of this side-reading antibody alone will provide adequate steric hindrance to block the binding of GLP-1(7-37) to the GLP-1 receptor has not been tested, but the fact that the antibody inhibits the insulin-releasing action of GLP-1(7-36)amide in the isolated perfused rat pancreas suggests that it would also block the action of GLP-1(7-37). The two antibodies together will be expected to show a higher affinity for GLP-1(7-36)amide and GLP-1(1-36)amide than either alone, possibly one or two orders of magnitude higher than their individual binding constants of about 10^7 L/mol.[26]

Although theoretical calculations show that at a K_a value of 1.5 to 1.6 $\times$ 10^7 L/mol the antibody concentrations achieved in the rats ($\sim 10^{-6}$ mol/L) would be sufficient to reduce free GLP-1(7-36)amide concentrations by about 95% over the physiological range, the measured binding of labeled GLP-1(7-36)amide by antibody-containing plasma from individual rats was lower, at a mean of 76.9%. However, such measurements assume that the antibodies have the same affinity for labeled peptide as for unlabeled peptide, which may not be true, and also assume that the charcoal separation process does not strip a proportion of the labeled peptide from the antibodies, a process that is particularly likely to occur with antibodies of relatively low affinity such as these. Thus, while the label-binding results show that the antibody-containing plasma was indeed capable of binding about three fourths of the labeled GLP-1(7-36)amide presented, this must be regarded as a minimum estimate of hormone binding, and actual values may be somewhat higher.

The plasma concentration of amidated GLP-1 in the control experiments showed the expected response to intragastric glucose. However, in the presence of the antibodies, high basal and stimulated levels (>100 pmol/L) of amidated GLP-1 were found in the extracted plasma samples, precluding the detection of a response. It is presumed that these high levels result from the cumulative binding of endogenous amidated forms of GLP-1 subsequent to injection of the antibodies, the sum total of which is released from the antibodies by the extraction procedure. This total would reflect the cumulative secretion of endogenous GLP-1 during the periods concerned, but the present data do not allow any speculation as to whether the basal secretion rate would be augmented by any compensatory feedback mechanism provoked by reducing the circulating levels of free hormone. There is no reason to suppose that the presence of the antibodies per se diminishes the secretion of GLP-1 in response to glucose. Since neither antibody cross-reacts significantly with GIP, one of the important stimuli to GLP-1 release from the intestine should be unaffected, and similarly, there is no special reason to suppose that nervous or luminal stimuli of intestinal GLP-1 release should be impaired by the antibodies.

The effect of the antibodies on the increase in plasma glucose after intragastric glucose gavage was unexpected. For this reason, sample identification was verified and the analysis was repeated by an independent operator, with the same results. In the presence of the antibodies, the increase in plasma glucose was significantly allowed. This contrasts with results obtained by GLP-1 receptor blockade with exendin(9-39)amide, when glycemia is augmented, an effect attributed to reduction of the insulin response[10] and possibly also to blockade of peripheral effects of GLP-1 on glucose disposal.[27] The results also contrast with the increased levels of blood glucose found after oral glucose challenge in GLP-1 receptor gene knockout mice.[28] However, the normal body weight and feeding behavior of these mice serve to illustrate how other mechanisms may override the effects of a congenital lack of the GLP-1 receptor. The mechanism of the reduction in glucose elevation in the present study requires further elucidation. It may be due to an unpredictable pharmacological action of the antibodies unrelated to their GLP-1 binding, or it may imply an effect of GLP-1 to promote glucose absorption from the gut by mechanisms independent of exendin(9-39)amide-blocked receptors but nevertheless blocked by antibody attachment to the active peptides. In this respect, it is interesting that a monoclonal antibody reported to react with both GLP-1 and GLP-2 blocked the glucose-induced increase in

maximal carrier-mediated glucose uptake by rat jejunal basolateral membrane vesicles.[29] This pointed to a role for the glucagon-like peptides in upregulating GLUT-2 activity, but prior infusion of the rats with either GLP-1 or GLP-2 attributed this effect to GLP-2. However, the antibodies used in the present study do not cross-react with GLP-2.

Although the slower increase of plasma glucose in the present experiments would have contributed to the reduction of the insulin response, it seems inadequate to explain the near-total abolition of any plasma insulin elevation during the first 10 minutes after glucose gavage. The plasma glucose elevation at 10 minutes in the presence of antibodies was about equal to the increase at 5 minutes in the control experiments, in which there was already a marked increase in plasma insulin. Furthermore, the level and rate of increase of plasma glucose in the presence of the antibodies were comparable to the values found to permit stimulation of insulin release by GLP-1 in the isolated perfused rat pancreas.[30]

REFERENCES

1. Kreymann B, Williams G, Ghatei MA, et al: Glucagon-like peptide-1 (7-36): A physiological incretin in man. Lancet 2:1300-1304, 1987
2. Herrmann C, Göke R, Richter G, et al: Glucagon-like peptide-1 and glucose-dependent insulin-releasing polypeptide plasma levels in response to nutrients. Digestion 56:117-126, 1995
3. Herrmann-Rinke C, Voge A, Hess M, et al: Regulation of glucagon-like peptide-1 secretion from rat ileum by neurotransmitters and peptides. J Endocrinol 147:25-31, 1995
4. Dumoulin V, Dakka T, Plaisancie P, et al: Regulation of glucagon-like peptide-1-(7-36)amide, peptide YY, and neurotensin secretion by neurotransmitters and gut hormones in the isolated vascularly perfused rat ileum. Endocrinology 136:5182-5188, 1995
5. Brubaker PL: Regulation of intestinal proglucagon-derived peptide secretion by intestinal regulatory peptides. Endocrinology 128:3175-3182, 1991
6. Mojsov S, Weir GC, Habener JF: Insulinotropin: Glucagon-like peptide 1 (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 79:616-619, 1987
7. Holst JJ, Ørskov C, Nielsen OV, et al: Truncated glucagon-like peptide 1, an insulin-releasing hormone from the distal gut. FEBS Lett 211:169-174, 1987
8. Weir GC, Mojsov S, Hendrick GK, et al: Glucagonlike peptide 1 (7-37) actions on endocrine pancreas. Diabetes 38:338-342, 1989
9. Göke R, Fehmann H-C, Linn T, et al: Exendin-4 is a high potency agonist and truncated exendin-(9-39)-amide an antagonist at the glucagon-like peptide 1-(7-36)-amide receptor of insulin-secreting beta-cells. J Biol Chem 268:19650-19655, 1993
10. Kolligs F, Fehmann HC, Göke R, et al: Reduction of the incretin effect in rats by the glucagon-like peptide 1 receptor antagonist exendin (9-39) amide. Diabetes 44:16-19, 1995
11. Valverde I, Morales M, Clemente F, et al: Glucagon-like peptide 1: A potent glycogenic hormone. FEBS Lett 349:313-316, 1994
12. Ruiz-Grande C, Alarcón C, Mérida E, et al: Lipolytic action of glucagon-like peptides in isolated rat adipocytes. Peptides 13:13-16, 1992
13. Suzuki S, Kawai K, Ohashi S, et al: Comparison of the effects of various C-terminal and N-terminal fragment peptides of glucagon-like peptide-1 on insulin and glucagon release from the isolated perfused rat pancreas. Endocrinology 125:3109-3114, 1989
14. Gefel D, Hendrick GK, Mojsov S, et al: Glucagon-like peptide-1 analogs: Effects on insulin secretion and adenosine 3′,5′-monophosphate formation. Endocrinology 126:2164-2168, 1990
15. van Delft J, Uttenthal LO, García-Hermida O, et al: Identification of amidated forms of GLP-1 in rat tissues using a highly sensitive radioimmunoassay. Regul Pept 70:191-198, 1997
16. Varndell IM, Bishop AE, Sikri KL, et al: Localization of glucagon-like peptide (GLP) immunoreactants in human gut and pancreas using light and electron microscopic immunocytochemistry. J Histochem Cytochem 33:1080-1086, 1985
17. Suda K, Takahashi H, Fukase N, et al: Distribution and molecular forms of glucagon-like peptide in the dog. Life Sci 45:1793-1798, 1989
18. Namba M, Itoh H, Watanabe N, et al: Multiple forms of glucagon-like peptide-1 and glucagon-like immunoreactivities in canine gastrointestinal tract and their release into circulation. Biomed Res 11:247-254, 1990
19. Eissele R, Göke R, Willemer S, et al: Glucagon-like peptide-1 cells in the gastrointestinal tract and pancreas of rat, pig and man. Eur J Clin Invest 22:283-291, 1992
20. Abello J, Ye F, Bosshard A, et al: Stimulation of glucagon-like peptide-1 secretion by muscarinic agonist in a murine intestinal endocrine cell line. Endocrinology 134:2011-2017, 1994
21. Gill SS, Lee YC, Ghatei MA, et al: The use of a rat isolated ileal preparation to investigate the release of neurotensin. Clin Exp Pharmacol Physiol 11:457-466, 1984
22. Plaisancie P, Bernard C, Chayvialle JA, et al: Regulation of glucagon-like peptide-1-(7-36)amide secretion by intestinal neurotransmitters and hormones in the isolated vascularly perfused rat colon. Endocrinology 135:2398-2403, 1994
23. Roberge JN, Brubaker PL: Regulation of intestinal proglucagon-derived peptide secretion by glucose-dependent insulinotropic peptide in a novel enteroendocrine loop. Endocrinology 133:233-240, 1993
24. Roberge JN, Gronau KA, Brubaker PL: Gastrin-releasing peptide is a novel mediator of proximal nutrient-induced proglucagon-derived peptide secretion from the distal gut. Endocrinology 137:2383-2388, 1996
25. Buchan AM, Polak JM, Capella C, et al: Electron immunocytochemical evidence for the K cell localization of gastric inhibitory polypeptide (GIP) in man. Histochemistry 56:37-44, 1978
26. Siddle K: Properties and applications of monoclonal antibodies, in Collins WP (ed): Alternative Immunoassays. New York, NY, Wiley, 1985, pp 13-37
27. D'Alessio DA, Prigeon RL, Ensinck JW: Enteral enhancement of glucose disposition by both insulin-dependent and insulin-independent processes. A physiological role of glucagon-like peptide 1. Diabetes 44:1433-1437, 1995
28. Scrocchi LA, Brown TJ, MacLusky N, et al: Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med 2:1254-1258, 1996
29. Cheeseman CI, Tsang R: The effect of GIP and glucagon-like peptides on intestinal basolateral membrane hexose transport. Am J Physiol 271:G477-G482, 1996
30. Jia X, Brown JC, Ma P, et al: Effects of glucose-dependent insulinotropic polypeptide and glucagon-like peptide-I-(7-36) on insulin secretion. Am J Physiol 268:E645-E651, 1995