Effect of glucagon-like peptide 1 (GLP-1) antibodies on glucose-induced insulin secretion in rats

KENJI SHIMA, CHIZUKO OHBOSHI and MEISEI HIROTA
Department of Laboratory Medicine, School of Medicine, The University of Tokushima, Tokushima 770, Japan

ABSTRACT
The effects of glucagon-like peptide-1 (GLP-1) antiserum on glucose tolerance and insulin secretion were examined in un-restrained, un-anesthetized rats. In antibody-treated rats the plasma glucose, immunoreactive insulin (IRI) and glucagon immunoreactivity levels were similar to those of control rats treated with normal rabbit serum (NRS) in the starved state. In control experiments, the same amount of anti-GLP-1 immunoglobulins significantly reduced the insulinotropic effect of exogenous GLP-1(7-36 amide) infused at a rate of 50 ng/kg/min together with glucose (6.3 mg/kg/min). No significant difference was found in the plasma glucose or IRI levels or the respective 120-min integrated areas after an oral glucose load (1 g/kg) in animals treated with GLP-1 antibodies and control animals treated with NRS (IRI: 8.5±1.6 nmol/l min in controls vs. 6.4±1.2 nmol/l min in antibody-treated rats). After an oral glucose load, the level of plasma GLP-1 immunoreactivity (IR GLP-1) decreased slightly, but significantly, from the basal level of 197±11 pmol/l to a nadir of 160±9.7 pmol/l at 90 min in control rats. The plasma glucose and IRI levels after an intraduodenal glucose load (1 g/kg) did not differ significantly in the group treated with GLP-1 antiserum and the control group. However, the 120-min integrated IRI response to the glucose load was significantly less in the group treated with GLP-1 antiserum than in the control group (5.0±1.1 nmol/l min vs. 18.6±2.9 nmol/l min, P<0.005). The plasma IR GLP-1 level was significantly increased after an intraduodenal glucose load from 145±15 pmol/l in the basal state to a peak after 90 min of 191±22 pmol/l.

The main candidate for incretin, a hormonal factor in the gut that enhances nutrient disposal, has been thought to be gastric inhibitory polypeptide (GIP), which enhances glucose-stimulated insulin release in man (1, 2, 4, 11). However, administration of GIP antibodies does not abolish the incretin effect (3), implying that other factors are involved. Recently, glucagon-like peptide 1 (GLP-1), especially a smaller form of GLP-1, GLP-1(7-36 amide), was found to have a potent effect in enhancing glucose-induced insulin release at physiological concentrations in humans (7), pigs (6), and rats (8, 12). The effective concentration of GLP-1(7-36 amide) for a similar effect to GIP on glucose-stimulated insulin release was reported to be as low as one-tenth of that of GIP under the same experimental conditions (12), indicating that GLP-1(7-36 amide) has a greater insulinotropic effect than GIP. Furthermore, GLP-1(7-36 amide) is released from the gut after an oral glucose load (6). These findings suggest that this peptide may function as an incretin.
To determine the importance of GLP-1 in the control of insulin release and glucose tolerance of rats after oral or intraduodenal administration of glucose, we studied the effect of experimental GLP-1 deficiency (obtained by the injection of anti-GLP-1 serum) in un-restrained, un-anesthetized rats.

GLP-1 antiserum Antiserum was raised in rabbits as reported previously (15). Of the antisera obtained, LMT-02 was used in this experiment. The immunological characteristics of LMT-02 are as shown below.

An immunoglobulin fraction of LMT-02 was prepared by ammonium sulphate precipitation and the immunoglobulin fraction was reconstituted with saline (0.154 M). An immunoglobulin fraction of normal rabbit serum (NRS) was prepared similarly. The avidity to GLP-1 and binding capacity of the resulting anti-GLP-1 immunoglobulin pool, assessed by Scatchard analysis, were $3.26 \times 10^9$ M$^{-1}$ and $5.46 \times 10^{-6}$ M, respectively. LMT-02 reacted equally with GLP-1(1–37) and GLP-1 (7–36 amide). It did not cross-react with insulin, gastrin, secretin, GIP or vasoactive intestinal polypeptide (VIP) and show little cross-reactivity (0.001%) with pancreatic glucagon.

Experimental procedure Male Wistar rats weighing 300–350 g were used for experiments. The animals were starved overnight and then anesthetized by intraperitoneal injection of sodium pentobarbital (Somnopentyl, Pitman-Moore, NG, U.S.A.) at a dose of 60 mg per kg body weight. The right jugular vein was exposed and a small polyethylene catheter was inserted 1.7 cm into the vein and ligatured in place. The distal end of the catheter was brought out in the dorsal region of the neck. The catheter was kept patent by infusing isotonic saline with a Holter pump (Critikon, FL, U.S.A.). Postoperatively, the rats were allowed to recover in separate cages. The experiment was started several hours after the operation when all the animals had recovered from anesthesia and were alert. Blood samples (1 ml) were withdrawn into heparinized syringes from the indwelling catheter and promptly transferred to plastic tubes containing 500 U of aprotinin and EDTA-2Na at 1.2 mg/ml of blood. Plasma samples were stored at $-20^\circ$C until assays for immunoreactive insulin (IRI), IR GLP-1 and GI.

![Graph](image.jpg)

Fig. 1 Changes in the plasma levels of glucose (BS), immunoreactive insulin (IRI) and glucagon immunoreactivity (GI) in starved rats after intravenous injection of anti GLP-1 immunoglobulins (LMT, ○--○, n=6) or normal rabbit immunoglobulins (NRS, □--□, n=6).

Chemical analyses Free GLP-1 and glucagon were extracted by the method of Nakagawa et al. (9). Plasma samples collected from test animals after LMT administration were incubated with freshly prepared $^{125}$I-GLP-1 (1–37) and the titer and percentage binding of unbound antibodies were measured. Plasma glucose was measured by a glucose oxidase method (Fuji Dri-chem., Tokyo, Japan). The plasma IRI concentration was measured by double-antibody radioimmunoassay with a commercial kit (Eiken Immunochemical, Tochigi, Japan) with rat insulin as a standard. IR GLP-1 was assayed with specific anti-GLP-1 serum (LMT-01) as reported elsewhere (13, 15). GI was measured by double-antibody RIA.
with C-terminal reactive OAL-123 antiserum as described previously (14).

Analysis of variance and the Wilcoxon matched-pairs singed-ranks test were used for statistical analysis. Data in the text and figures are presented as means or means±SEM.

**Injection of GLP-1 antiserum into starved rats (Experiment 1)** Blood samples were taken from 6 rats that had been starved and 15 min later the animals were given a bolus injection of 60 μl of anti-GLP-1 immunoglobulin (LMT). Further blood samples were withdrawn 0, 30, 60, 90 and 120 min later. Blood samples were also collected according to the same schedule from 6 control rats treated with 60 μl of NRS in place of LMT.

As shown in Fig. 1, no significant change in the plasma level of glucose, IRI or GI was observed during the test period in either the antibody-treated or the control group. Antibody was present in excess throughout the 120-min period, as determined by the percentage binding to 125I-GLP-1 (range 83–95% bound/total).

**Injection of GLP-1 antiserum before application of exogenous GLP-1 (Experiment 2)** Group I of 8 control rats received intravenous glucose infusion at a rate of 6.3 mg/kg/min. The intravenous glucose load was combined with infusion of 50 ng of GLP-1 (7-36 amide)/kg/min in group 2 of 7 rats and group 3 of 10 rats. Group 3 received a bolus injection of 60 μl of LMT and groups 1 and 2 received 60 μl of NRS 15 min before the glucose infusion. Blood samples were taken just before the bolus injection of LMT or NRS (−15 min), and before (0 min) and 30, 60, 90, and 120 min after the start of glucose infusion.

As shown in Fig. 2, the decrease in blood glucose induced by exogenous GLP-1(7-36 amide) was completely counteracted by GLP-1 antiserum. Similarly, the enhancement of increase in the IRI level by GLP-1(7-36 amide) was reversed by GLP-1 antiserum to exactly that observed after treatment with glucose alone. The 120-min integrated responses of plasma glucose and IRI to exogenous administration of GLP-1(7-36 amide) differed in the control and antibody-treated animals (Table 1). The plasma IR GLP-1 levels at 90 min (148±14.4 pmol/l) and 120 min (142±11.9 pmol/l) in the rats treated with glucose alone were significantly lower than the basal level (189±14.4 pmol/l). On the contrary, the plasma IR GLP-1 level increased significantly from the basal level of 168±28.8 pmol/l to a peak of 192±28.8 pmol/l at the end of the experiment in rats infused with glucose and GLP-1(7-36 amide). Antibody was present in excess throughout the experiment and at 120 min the percentage binding of 125I-GLP-1 was 81% of the total counts.

**Injection of GLP-1 antiserum before the oral glucose load (Experiment 3)** A group of 16 rats was starved overnight, blood was taken, and 15 min later 60 μl of LMT was injected.

![Fig. 2 Effect of immunoneutralization of exogenous GLP-1(7-36 amide) on plasma levels of glucose (BS), IRI and IR GLP-1. Rats received a bolus injection of normal rabbit immunoglobulin and glucose infusion at a rate of 6.3 mg/kg/min ( –●–, n=8), normal rabbit immunoglobulin, glucose and GLP-1(7-36 amide) infusion at a rate of 50 ng/kg/min ( –●–, n=7) or anti-GLP-1 immunoglobulins, glucose and GLP-1(7-36 amide) ( –––, n=10). Results were means±SEM. Significance of differences between control and test groups: *, P<0.05; ***, P<0.025; *, significant difference from the value in the basal state.](image-url)
Table 1 120-min Integrated Responses of Plasma Glucose and IRI to Exogenous Administration of GLP-1(7–36 Amide) or to an Intraduodenal Glucose Load in Rats with or without GLP-1 Antiserum Treatment

<table>
<thead>
<tr>
<th></th>
<th>Glucose (nmol/l min)</th>
<th>IRI (nmol/l min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiment 2</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose alone</td>
<td>332.3±34</td>
<td>9.02±1.2</td>
</tr>
<tr>
<td>NRS</td>
<td>198.2±26.5</td>
<td>18.6±3.6</td>
</tr>
<tr>
<td>LMT</td>
<td>295.1±27.8</td>
<td>7.4±1.4</td>
</tr>
<tr>
<td><strong>Experiment 4</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NRS</td>
<td>100.4±22.8</td>
<td>18.6±2.9</td>
</tr>
<tr>
<td>LMT</td>
<td>109.5±21.5</td>
<td>5.0±1.1</td>
</tr>
</tbody>
</table>

Experiment 2: a bolus dose of GLP-1 antiserum (LMT) or normal rabbit immunoglobulin (NRS) was injected 15 min before infusion of glucose (6.3 mg/kg min) alone (glucose alone) or together with GLP-1(7–36 amide) (50 ng/kg min). Experiment 4: a bolus dose of GLP-1 antiserum (LMT) or normal rabbit immunoglobulin (NRS) was injected 15 min before an intraduodenal glucose load (1 g/kg). See the text for details. \(^{a}\)vs. NRS (\(P<0.01\)); \(^{b}\)vs. NRS (\(P<0.025\)); \(^{c}\)vs. NRS (\(P<0.05\)); \(^{d}\)vs. NRS (\(P<0.005\))

intravenously. At 0 min, 20% glucose solution was given orally at a dose of 1 g per kg body weight through a small-bore syringe, and blood samples were collected at 0, 30, 60, 90 and 120 min. The control group of 16 rats received 60 \(\mu\)l of NRS instead of LMT, and blood sampling and oral glucose loading were carried out as in the antibody-treated group.

Oral glucose administration resulted in a significant increase in plasma IRI concentration in both the control group treated with normal rabbit immunoglobulins and the test group treated with GLP-1 antibodies, and there was no significant difference in the plasma IRI levels in these two groups (Fig. 3). The 120 min integrated IRI response to the glucose load also did not differ significantly in the control and antibody-treated groups (8.5±1.6 nmol/l min for controls vs. 6.4±1.2 nmol/l min for the antibody-treated group). The glucose tolerance curves of the two groups were superimposable, implying no change in glucose tolerance by treating the animals with LMT. After an oral glucose load, the plasma IR GLP-1 concentration decreased slightly, but significantly from the basal level (197±11 pmol/l) to a nadir of 160±9.7 pmol/l at 90 min in the control group. An excess binding capacity of the antibodies was maintained throughout the experiment.

Injection of GLP-1 antiserum before an intraduodenal glucose load (Experiment 4) Groups of 8 experimental and control rats were used. The procedure was essentially as for Experiment 3, but 20% glucose solution was injected intraduodenally at a dose of 1 g per kg body weight through a catheter which had been inserted into the duodenum and ligated under laparotomy several days previously.

The plasma glucose and IRI levels after the intraduodenal glucose load did not differ significantly in the control group and the group treated with GLP-1 antiserum (Fig. 4). However, the 120 min integrated IRI response to the glucose load was significantly smaller in the rats treated with GLP-1 antiserum than in those treated with NRS (Table 1). There was no significant difference in the integrated plasma glucose response to the intraduodenal glucose load in the two groups. The plasma IR GLP-1 level was significantly increased from a basal level of 145±15 pmol/l to a peak 90 min after the intraduodenal glucose load of 190±22 pmol/l. Antibodies were present in excess of their binding capacity throughout the experimental period.

If truncated GLP-1 is an incretin, injection of GLP-1 antiserum before OGTT, which neutralizes endogenous GLP-1, should reduce insulin output and decrease glucose tolerance after an oral glucose load. However, in the present study we found that neutralization of endogenous GLP-1 by anti-GLP-1 serum does not decrease insulin release after an oral glucose load. This inability of the antibody to
modify glucose-stimulated insulin secretion could not be attributed to incomplete neutralization of endogenous truncated GLP-1, because excess antibodies were present throughout the test period. The fact that the stimulatory effect of GLP-1(7-36 amide) on insulin release was significantly suppressed when the animals were treated with anti-GLP-1 serum before injection of the test agent, indicates that the antisera used in the present study binds GLP-1(7-36 amide) and neutralizes its biological activity in vivo.

However, the significance of GLP-1 as an incretin in rats could not be assessed in the OGTT study, because no elevation of plasma IR GLP-1 was observed after the glucose load, though Kreymann et al. (7) demonstrated an increase in the plasma level of GLP-1(7-36)-like immunoreactivity after oral glucose and after a test breakfast in man. Plasma IR GLP-1 consists of GLP-1(1-37), probably originating from the pancreas, as well as truncated GLP-1, the intestinal form of GLP-1. Moreover, the circulating IR GLP-1 reflects the relative proportions of pancreatic GLP-1 and intestinal GLP-1, which in turn is influenced by various conditions, such as an oral glucose load that decreases pancreatic GLP-1, but increases intestinal GLP-1 (5, 10). When the anti-GLP-1 serum used in radioimmunoassay is not specific to truncated GLP-1 but also reacts with GLP-1(1-37), like our LMT-01, both forms of GLP-1 are measured as IR GLP-1 and the total plasma IR GLP-1 reflects the sum of these two components. This might be one reason why a slight decrease, rather than elevation, in
plasma IR GLP-1 was observed after an oral glucose load in the present study. The presence of an incretin effect in rats was demonstrated by Ebert et al. (3) who showed that at nearly identical blood glucose levels, the insulin output due to intraduodenal glucose was approximately twice that after intravenous glucose infusion. Even under these conditions, we could not definitely confirm that GLP-1 was an incretin, because administration of GLP-1 antiserum resulted in no significant reduction of the plasma IRI response to an intraduodenal glucose load in un-restrained, un-anesthetized rats. However, the integrated insulin output was significantly different with and without GLP-1 antiserum. Furthermore, unlike an oral glucose load, an intraduodenal glucose load increased the plasma IR GLP-1 level to a similar extent to that elicited by exogenous administration of GLP-1(7-36 amide) at a rate of 50 ng/kg/min.

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REFERENCES