Portal GLP-1 administration in rats augments the insulin response to glucose via neuronal mechanisms

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Received 28 February 2000; accepted in final form 31 May 2000

GLUCAGON-LIKE PEPTIDE-1 (GLP-1) is a potent incretin that is released from the distal small intestine in response to absorbed nutrients. Its primary function is believed to be the sensitization of the β-cells in the islets of Langerhans to stimulation by glucose. GLP-1 is released by the L cells in the intestinal tract as GLP-1-(7—36) amide, which is derived from proglucagon (8, 10). GLP-1 is rapidly inactivated by the ubiquitous enzyme dipeptidyl peptidase IV (DPP-IV) (4). This enzyme clips two penultimate amino acids and renders GLP-1-(9—36) amide, which is inactive or even has antagonistic properties, depending on the tissue (16). The inactivation of GLP-1 by DPP-IV is very rapid, leading to a half-life of the active peptide of ~1—1.5 min in pigs, thereby exceeding the cardiac output rate by a factor of 2 (11). Furthermore, it has recently been demonstrated that 50% of GLP-1-(7—36) amide that is released from the intestinal L cells is degraded in the surrounding capillaries (9). Thus the active GLP-1-(7—36) amide may never reach the pancreas. Therefore, we hypothesized that GLP-1 released by the L cells may trigger an intermediatory mechanism locally that delays the signal to the pancreas. A recent study reported that GLP-1 administered via the hepatic portal vein will increase the firing rate of hepatic afferent nerves and, in a reflex manner, also increase the activity of the pancreatic vagal fibers (12). This led us to hypothesize that the hepatic portal system may constitute one site of GLP-1 action. The goal of this study was to investigate whether an indirect mechanism triggered in the hepatoportal circulatory system may be involved in the stimulation of insulin secretion by endogenously secreted GLP-1. To that end, we mimicked the appearance of glucose and GLP-1 in the portal system and studied whether the augmentation of the insulin response would be affected by specific blockade of parts of the autonomic nervous system. We further studied whether administration of GLP-1 to the pancreas via the jugular vein would be similarly affected by autonomic blockade.

METHODS

Animals and Surgery. Male Sprague-Dawley rats (Hilltop, PA) were maintained on normal light cycle (lights on 6:00 AM to 6:00 PM) with ad libitum access to tap water and standard rodent chow (Purina Labs, Richmond, IN). The animals were aseptically implanted with a Silastic catheter in the right jugular vein under ketamine-Rompun-acepromazine anesthesia. The catheter was externalized in the nape of the neck and was filled with a solution of heparin and polyvinylpyrrolidone. A second Silastic catheter was implanted into the hepatic portal vein ~8 mm caudal of the liver. This method has been described in detail previously (17). The animals were allowed to recover until they reached their preoperative weight again, but a minimum of 5 days was allowed even when weight recovery was achieved earlier.

Reagents. GLP-1-(7—36) amide was obtained from Bachem (Torrance, CA). The ganglionic blocker chlorisondamine (CS) chloride (Ciba Pharmaceuticals, Summit, NJ) was administered at 3 mg/kg as an intravenous (jugular vein) bolus. Methyl atropine (atropine methyl bromide supplied by Sigma, St. Louis, MO), a peripherally acting muscarinic blocker, was injected into the jugular vein at 0.5 mg/kg. Glucose was administered into the portal vein at 500 mg/kg. All doses were given as separate injections.

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Protocols. The animals were fasted starting at 5:00 PM on the day before the experiment. At \( t = -30 \) min on the day of the experiment, the cannulas were connected to sampling tubing. At \( t = 0 \) and \( t = 30 \) min, a 200-μL bolus of vehicle (saline with 1% BSA) or GLP-1 (10 pmol/kg) was injected into the portal or jugular vein. Glucose (500 mg/kg, 1 ml/kg) was injected into the portal vein at \( t = 30 \) min, immediately before the second vehicle or GLP-1 administration. In some experiments, a ganglionic blocker (CS-HCl, 3 mg/kg, \( t = -30 \) min) or a peripheral muscarinic blocker (0.5 mg/kg methyl atropine, \( t = -10 \) and \( t = 20 \) min) was administered intravenously before portal injections.

Blood samples were obtained through the jugular vein catheter. Two basal samples were withdrawn at \( t = -10 \) and \( t = 0 \) min, and additional samples were withdrawn over 90 min. All samples were replaced by donor blood containing citrate and sodium citrate as anticoagulant. Blood samples were collected into chilled Eppendorf tubes containing EDTA and trasylol to achieve final concentrations of 2.5 mg EDTA and 1,000 kallikrein inhibitory units of trasylol per milliliter of blood. Samples were centrifuged, and plasma was stored at \(-20^\circ C\) until analyses. The protocol was reviewed and approved by the Novartis Animal Care and Use Committee.

**Plasma analyses.** Plasma glucose was analyzed using a modified Sigma Diagnostics glucose oxidase kit (Sigma). Plasma immunoreactive insulin levels were assayed by a double-antibody RIA method using a specific anti-rat insulin antibody from Linco Research (St Louis, MO). The assay has a lower limit of detection of 30 pmol/l with intra- and inter-assay variations of <5%.

Data are expressed as means ± SE. Incremental integrated areas under the curve were calculated by the trapezoidal rule. Statistical analysis was performed by \( t \)-test. All statistical significance reflects two-tailed testing unless stated otherwise.

**RESULTS**

Portal GLP-1 (10 pmol/kg, \( n = 9 \)) augmented the integrated insulin response to a portal glucose bolus (Fig. 1A). This near doubling of the insulin response to portal glucose (Table 1) was in marked contrast to the relatively minor stimulation of insulin secretion induced by portal GLP-1 alone (Fig. 1B). Administration of GLP-1 into the right atrium together with the portal glucose load was equally efficacious in augmenting the insulin response (Fig. 2A). Indeed, portal and jugular vein-injected GLP-1 had nearly identical effects on the glucose excursion after the portal glucose challenge (Fig. 2B). This suggests that the increased glucose disposal rate found after GLP-1 administration (Table 1) may be a consequence of the increased insulin response. The only difference found between groups of animals receiving GLP-1 in the portal vein versus the

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**Table 1. Basal immunoreactive plasma IRI and glucose levels, glucose disappearance rates, and integrated insulin responses to portal glucose in rats receiving combinations of GLP-1 and nervous blockers**

<table>
<thead>
<tr>
<th></th>
<th>( n )</th>
<th>Basal Plasma G, mM</th>
<th>Disappearance Rate of G, %/min</th>
<th>Basal Plasma IRI, pmol/l</th>
<th>IRI-AUC, pmol/l×10  min</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>8</td>
<td>5.3 ± 0.3</td>
<td>2.70 ± 0.3</td>
<td>55.5 ± 10.6</td>
<td>6975 ± 716</td>
</tr>
<tr>
<td>IPV GLP-1 + G</td>
<td>9</td>
<td>4.9 ± 0.1</td>
<td>3.52 ± 0.20*</td>
<td>57.3 ± 8.2</td>
<td>12650 ± 1455†</td>
</tr>
<tr>
<td>CS + IPV GLP-1 + G</td>
<td>6</td>
<td>4.9 ± 0.1</td>
<td>2.93 ± 0.26</td>
<td>32.1 ± 5.8</td>
<td>7786 ± 851§</td>
</tr>
<tr>
<td>Atr + IPV GLP-1 + G</td>
<td>7</td>
<td>5.0 ± 0.2</td>
<td>2.85 ± 0.44</td>
<td>39.4 ± 8.0</td>
<td>12234 ± 1898*</td>
</tr>
<tr>
<td>IV GLP-1 + G</td>
<td>6</td>
<td>5.5 ± 0.2</td>
<td>3.53 ± 0.28 (( P = 0.074 ))</td>
<td>60.9 ± 9.0</td>
<td>13124 ± 1617†</td>
</tr>
<tr>
<td>CS + IV GLP-1 + G</td>
<td>7</td>
<td>4.9 ± 0.2</td>
<td>3.34 ± 0.23</td>
<td>20.6 ± 3.5*</td>
<td>12445 ± 295‡</td>
</tr>
<tr>
<td>CS + G</td>
<td>5</td>
<td>4.9 ± 0.0</td>
<td>3.12 ± 0.26</td>
<td>24.7 ± 7.2 (( P = 0.06 ))</td>
<td>8562 ± 683</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), nos. of rats. IRI, immunoreactive insulin concentrations; GLP-1, glucagon-like peptide-1; G, glucose; AUC, area under the curve; IPV, administered into hepatoporal vein; IV, administered into the jugular vein; CS, chlorisondamine; Atr, methyl atropine.

*\( P < 0.05 \), †\( P < 0.01 \) vs. G alone; ‡\( P < 0.05 \) vs. IPV GLP-1 + G (\( P \) values given as text always vs. G alone).
jugular vein was observed when the peptide was administered in the absence of the glucose load. The insulin response to GLP-1 alone was much larger when GLP-1 was administered via the jugular vein than when administered via the portal vein (Fig. 2C). This finding supports the expectation that less GLP-1 would reach the pancreas after portal administration than after injection into the jugular vein. To reconcile the profound augmentation of glucose-induced insulin secretion after portal administration, we hypothesized that the effects of portal GLP-1 are, at least in part, mediated by the autonomic nervous system. Therefore, we repeated the study after complete ganglionic blockade with CS. Pretreatment with CS reduced the insulin response to portal GLP-1 and glucose to that seen with portal glucose alone (Fig. 3A). Concomitantly, ganglionic blockade reduced the glucose disappearance rate (Table 1).

Administration of the muscarinic blocker methyl atropine did not affect the augmentation of the insulin response to portal glucose by portal GLP-1 (Table 1). The glucose disappearance rates in the atropine-treated animals displayed a large degree of variability, which makes it impossible to ascertain whether there was an effect (Table 1).

In contrast to the actions of ganglionic blockade on the effects of portal GLP-1, the potentiation of insulin secretion by GLP-1 given in the jugular vein was not affected by CS (Fig. 3B and Table 1). Ganglionic blockade alone did not affect the insulin response to portal glucose (Fig. 4).

**DISCUSSION**

The purpose of this study was to investigate whether GLP-1 administered into the hepatic portal vein can augment the insulin response to a portal glucose load mimicking glucose appearing from absorbed carbohydrates. Furthermore, this study investigated whether this augmentation of insulin secretion is direct or indirect in nature. The results demonstrate that the augmentation of the insulin response to portal glucose is profound and much larger than the stimulation by portal GLP-1 administration in the absence of exogenous glucose. This finding is in accordance with the generally accepted view that GLP-1 sensitizes β-cells to stimulation by glucose (7, 19). However, the prevention of the augmentation of the portal GLP-1-induced insulin response by ganglionic blockade suggests that portal GLP-1 triggers a nervous mechanism. We further conclude that the failure to inhibit the portal GLP-1-induced augmentation of insulin secretion by the peripherally acting muscarinic blocker methyl atropine demonstrates that this mechanism does not involve the classic parasympathetic nervous system.
the effects of which are mediated by acetylcholine acting on muscarinic receptors. The data suggest that the effect of portal GLP-1 is mediated by nonmuscarinic parasympathetic activation or suppression of the sympathetic inhibitory tone.

We hypothesize that GLP-1 affects afferent nerves in the hepatoportal bed that lead to altered nervous control of the islets of Langerhans. Indeed, changes in firing rate of hepatic afferent and pancreatic efferent nerves have been demonstrated after administration of GLP-1 in the hepatoportal system (12). The slow and long-lasting nature of the effects in those studies may have been a consequence of the use of anesthesia and contrasts with the speed of the augmentation in the present study. The fact that the potentiation of the insulin response in the current study was discernible within 1 min after administration of glucose and GLP-1 suggests a nervous-nervous rather than nervous-humoral reflex loop.

The afferent signal is most likely transmitted by the afferent vagus nerve, considering the abundance of these fibers in this region. Furthermore, the vagal system has been demonstrated to be very sensitive to many gastrointestinal hormones and nutrients (13, 15). Several possibilities can be considered for the efferent part of the loop. Muscarinic parasympathetic stimulation of β-cells evokes a very strong secretory effect (in the presence of glucose) and has been shown to be part of the adaptation of the body to a number of metabolic challenges, including prolonged stimulation by glucose (3, 18). The lack of effect of methyl atropine rules out this hypothesis. Alternatively, the sympathetic nervous system may be involved in this process. Sympathetic nervous stimulation of the endocrine pancreas generally results in inhibition of insulin secretion (1), and the observed effects in the current study would suggest reduced sympathetic activity to the islets of Langerhans. This possibility remains to be investigated. However, the involvement of nonclassical neurotransmitters, e.g., neuropeptides, as yet an alternative explanation cannot be discarded. A number of peptides are potent cotransmitters in autonomic nerves in the pancreas (2) and may be responsible for the observed phenomenon.

It is likely that ganglionic blockade, or the altering of the balance between sympathetic and parasympathetic tone during atropine treatment, has some effect on circulatory parameters. However, the lack of effect of ganglionic blockade on the insulin response and glucose excursion after glucose administration without GLP-1 is evidence that any potential circulatory effect did not affect the pancreatic response. The lack of effect of ganglionic blockade on the stimulation of insulin secretion by glucose alone further rules out the possibility that portal glucose triggers a reflex that relies on the autonomic nervous system under the conditions of this experiment.

GLP-1 is one of the most potent stimulators of glucose-induced insulin secretion. Numerous studies have
investigated direct effects of GLP-1 on β-cells both in vitro and during continuous infusions in vivo. Endogenous GLP-1 is limited in its action by very rapid inactivation under influence of DPP-IV, which cleaves two NH₂-terminal amino acids, thereby rendering the hormone inactive (for its β-cell effect) (4). Fifty percent of the GLP-1-(7–36) amide released from the L cells is estimated to be inactivated in the capillary bed surrounding these cells (9). Furthermore, single pass through the liver inactivates a large fraction of the remaining active GLP-1 (>40%) (5). Thus these two processes together with inactivation in the circulatory system and in other organs can be expected to inactivate or remove most of the GLP-1 released from the intestine before the peptide can reach the pancreas in the active form.

Interestingly, peripheral administration of GLP-1 via the jugular vein also potentiated the insulin response to portal glucose. However, this response was not affected by ganglionic blockade. The larger insulin response to jugular GLP-1 than to portal GLP-1 in the absence of the glucose load is consistent with the expectation that more GLP-1 would reach the islets of Langerhans in the active form after administration into the jugular vein. Thus it appears that GLP-1 can affect insulin secretion both directly and via a nervous mechanism triggered in the hepatoportal system.

We conclude that portal GLP-1 greatly affects handling of a portal glucose load. This effect appears to be mediated by augmented insulin release. The data suggest that β-cell stimulation by portal GLP-1 is evoked via a neural reflex that may be triggered in the liver and is nonmuscarinic in nature. Because absorbed nutrients and GLP-1 first appear in the portal system, this mechanism may constitute a major pathway of GLP-1 action during meals. The effects of GLP-1 administration into the jugular vein do not appear to be mediated by the nervous system, suggesting that portal and peripheral GLP-1 may exert their effects on insulin secretion through different mechanisms.

Perspectives

The physiological significance of the redundancy between portal and direct augmentation of insulin secretion by GLP-1 is unclear at present but may reflect different regulatory mechanisms during large and small meals. A small meal triggering a small, transient release of GLP-1 may only be sufficient to trigger the nervous effects in the hepatoportal system. This would rapidly initiate the insulin response accelerating the transition of the liver from glucose production to glucose uptake (6). Only larger meals or slowly absorbed nutrients may lead to GLP-1 release in sufficient quantity and duration to affect the β-cells directly. Alternatively, it can be hypothesized that the GLP-1 receptors in the hepatoportal system and on β-cells constitute parts of a nervous loop mediating the endocrine response to endogenously released GLP-1. The well-documented insulinoic action of GLP-1 administered into the general circulation would then exert its effects through receptors normally responding to GLP-1 released by autonomic nerves. The support for this hypothesis is currently slim, and a recent report suggests that the GLP-1 receptor in the hepatoportal system and in the islets of Langerhans may differ as they show differential responsivity to GLP-1 agonists and antagonists of the exendin family (14). Due to its profound potency in augmenting glucose-stimulated insulin secretion, GLP-1 is likely to be an important mediator of prandial glucose homeostasis. Better understanding of the actions of endogenous GLP-1 and of the pathways by which this peptide acts will hopefully enable us to fully harness the beneficial actions for the treatment of diabetes.

The expert assistance of Lori Kwansik and Beverly Battle is gratefully acknowledged.

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