The Effects of GLP-1 on Insulin Release in Young and Old Rats in the Fasting State and During an Intravenous Glucose Tolerance Test

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Glucose intolerance is a common feature of the aging process, and aging per se is an etiologic factor for Type II diabetes mellitus. To characterize the beta cell abnormalities that occur with aging, we looked at the serum glucose and insulin levels of six young (3-month) and six old (22-month) Wistar rats at 0, 2, 4, 7, 10, 15, 20, and 30 minutes after an intravenous glucose load (IVGTT, 0.5g/kg glucose). We found that the fasting glucose and insulin levels were not significantly different between young and old rats. However, peak glucose levels were significantly higher in the old (349 ± 10 mg/dl) compared to the young (250 ± 7 mg/dl) animals (p < .0001). Insulin levels in the young animals peaked at 2 minutes (859 ± 171 pmol/l) with a quick return toward fasting levels by 7 minutes. The old animals had a delayed and blunted insulin response to glucose, achieving lower peak insulin levels (656 ± 164 pmol/l) 7 minutes after the glucose load. As insulin levels are also positively modulated by incretin hormones, we quantitated the fasting insulin responses of young and old animals to 0.5, 0.1, 0.2, 0.4, and 0.5 nmol/kg intravenous glucagon-like peptide-1 (GLP-1), the most potent incretin known. Insulin responses were similar in both age groups, with maximum insulin responses seen at 0.4 nmol/kg. GLP-1, in conjunction with the IVGTT, restored the acute insulin response to glucose and increased the clearance of glucose in the old animals. It therefore appears that old animals have an impaired glucose-mediated insulin release but maintain their insulin responsivity to GLP-1. This makes it a likely candidate in the treatment of Type II diabetes.

I MPAIRED glucose tolerance and Type II diabetes are well-described features of aging (De Fronzo, 1981; Zavaroni et al., 1986; Morley, 1987). Beta cell insensitivity to glucose and peripheral insulin resistance are suggested to be the underlying mechanisms (Reaven and Reaven, 1980; Olefsky, 1981). Various changes in insulin secretion have been described in aging and Type II diabetes (Palmer and Ensinck, 1975; Jackson, 1990). With both aging and Type II diabetes there appears to be a deficiency of the acute phase glucose-mediated insulin secretion from beta cells (Palmer and Ensinck, 1975) while late-phase insulin release appears intact (Cerasi et al., 1972). It has long been known that the gastrointestinal tract affects insulin release (Moore et al., 1906), but its role in the maintenance of glucose homeostasis in Type II diabetes is largely unknown. A class of gut hormones, known as incretins (Creutzfeldt and Ebert, 1993), are released into the blood in response to food and augment the insulin response to oral glucose. Several gut hormones have been suggested to be insulino-tropic, i.e., to augment insulin release. These include secretin, gastrin-releasing peptide, vasoactive intestinal peptide, and cholecystokinin. However, they are insulino-tropic only at pharmacologically important plasma concentrations. The most likely physiologically important incretins are glucose-dependent insulino-tropic hormone (GIP) and glucagon-like peptide-1 (GLP-1), with GLP-1 being the most potent of the two (Fehmann et al., 1992).

Beta cell sensitivity to GIP has been shown to decrease with age, with little or no change seen in nonmedicated diabetic subjects (Elahi et al., 1984). GLP-1, unlike GIP, has already been shown to lower blood glucose in Type II diabetic subjects in several studies (Gutniak et al., 1992; Nathan et al., 1992; Nauck et al., 1993), while its effects in aging per se have not yet been looked at. In addition, GLP-1 has recently been shown to be a potent inhibitor of gastric emptying in diabetes (Wills et al., 1996), and therefore some of its beneficial effects in diabetes could be attributed to a delaying of nutrient absorption, and not solely to it increasing insulin release. Wistar rats are known to develop glucose intolerance and to have significantly lower insulin responses to a glucose load with aging (Reaven et al., 1979). In a perfused pancreas model, their acute insulin response to glucose is severely depressed with age (Elahi et al., 1985). This is due to a reduction in the number of beta cells that respond to glucose in the early phase of stimulation by glucose (Perfetti et al., 1995). To characterize the effects of GLP-1 on age-dependent insulin release and glucose homeostasis, we investigated any alteration in beta cell responsivity to GLP-1 that might occur with aging in the fasted, anaesthetized Wistar rat. We then undertook an intravenous glucose tolerance test (IVGTT) in the presence and absence of GLP-1, delivered intravenously, and examined the serum glucose and insulin levels. This allowed us to interpret the results without the confounding variable of alterations in the rate of nutrient absorption.
MATeRIALS AND METHODS

Animals. — Three- and 22-month-old male Wistar rats were bred at the Gerontology Research Center of the National Institute on Aging and maintained on standard laboratory chow under a 12:12 h light-dark schedule. The animals were fasted from 4 p.m. on the day before every study.

Protocols. — On the study day, general anesthesia was induced by an intraperitoneal injection of pentobarbital (50 mg/kg). One cannula was placed in the femoral vein for infusions of GLP-1 (Bachem, King of Prussia, PA) and glucose, and another cannula was placed in the femoral artery for blood sampling. The artery was kept patent with flushes of saline containing 10 U/ml heparin. GLP-1 was infused over a 30-sec period in normal saline containing 0.1% BSA and 5% rat plasma. Blood samples (200 μl) were taken at -5, 0, 2, 4, 7, 10, 15, 20, and 30 min, centrifuged, and the serum stored at -20 °C. Two hundred μl normal saline was replaced after each blood draw.

GLP-1 concentration and IVGTT studies. — Six old and six young rats each received .05, 0.1, 0.2, 0.4, or 0.5 nmol GLP-1/kg. Each concentration was given to alternating young and old rats on the same day. An IVGTT (0.5 g/kg glucose) was given to six alternating young and old rats, also on the same day. On another day the IVGTT was repeated in young and old rats in conjunction with GLP-1 (0.4 nmol/kg). After 30 min the rats were killed by overdose with pentobarbital.

Assays. — Serum glucose concentration was estimated by the glucose oxidase method using a CCX Spectrum (Abbott, Irving, TX). Serum insulin levels were determined in the same samples as glucose by RIA using rabbit anti-human insulin antibody (Peninsula Laboratory, Belmont, CA) with 15,000-20,000 counts/min (cpm; 3.5-5 pmol) of 125I-insulin (Amersham, Arlington Heights, IL) and rat insulin as standard (Montrose-Rafizadeh et al., 1994; Perfetti et al., 1995).

Calculations and statistical analyses. — The mean concentrations of glucose and insulin were calculated for each time point during the IVGTTs and the bolus GLP-1 studies. The trapezoidal rule was used to calculate the area under the curve (AUC) over the time intervals shown in Figures 2 and 3. The K value (elimination rate constant) during the IVGTT was calculated between 0 and 15 min with log concentration of glucose vs time. All data are expressed as mean ± SE and analyzed by either unpaired Student’s t-test, one-way or two-way analysis of variance (ANOVA), as specifically indicated in the text.

RESULTS

GLP-1 concentration studies. — There was no significant difference between the young and old rats in the fasting serum glucose (122 ± 6.1 vs 116 ± 9.2 mg/dl; old vs young, respectively) or insulin (64 ± 6.4 vs 54.2 ± 9.6 pmol/l; old vs young, respectively). Insulin responses increased significantly at the 2 min time point in both young and old animals with each bolus (p < .001, for both age groups when compared to their respective basal levels) and had returned to baseline by 10 min. Maximum insulin response was seen in both young and old animals with 0.4 nmol/kg GLP-1. No further insulin release was seen with 0.5 nmol/kg GLP-1. No statistically significant difference between young and old rats was observed by using two-way analysis of variance of serum insulin levels after GLP-1 injection. Glucose levels were not consistently altered by the GLP-1 injections in either age.

IVGTT alone. — Serum glucose was significantly higher in the old animals compared to the young (Figure 2). Peak levels at 2 min were 349 ± 10 mg/dl vs 250 ± 7.0 mg/dl for the old vs the young, respectively (p < .0001). Thirty min after the IVGTT, serum glucose levels were back to baseline in the young (129 ± 7.4 mg/dl), but were still significantly higher in the old animals (156 ± 9.0 mg/dl; p = .01). Serum insulin levels peaked at 2 min in the young animals (859 ± 800 pmol/l) and

Figure 1. Dose-response (top panel, 2-min sample) and time course (bottom panel) of insulin levels attained after a bolus GLP-1 delivered intravenously to fasted, anaesthetised young and old rats. Each bar represents the mean ± SEM of 6 animals in each group. Time course was evaluated with a bolus of GLP-1 (0.5 nmol/kg), one representative concentration of five administered. Blood was drawn at the times plotted on the graph.

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**INSULIN RESPONSE TO GLP-1 IN AGING RATS**

**Figure 2.** Glucose levels in young (top panel) and old (bottom panel) rats after an IVGTT (0.5 g/kg glucose) in the presence or absence of a bolus of GLP-1 (0.4 nmol/kg). Each time point represents the mean ± SEM of 6 animals. Blood was drawn at the times plotted on the graph. In old rats (bottom panel), AUCo-t was 6,272 ± 215 for IVGTT alone vs 5,681 ± 129 mg/dl for IVGTT with 0.4 nmol GLP-1; *p* = .0164 by one-way ANOVA.

171 pmol/l; *p* < .001 when compared to basal; Figure 3) in parallel with the peak in glucose levels, whereas in the old animals at the very same time point the insulin only reached 384 ± 122 pmol/l (*p* = .04 when compared to basal). Insulin responses were clearly abnormal in the old animals (Figure 3). In the old animals peak insulin levels were not reached until 7 min after completion of the IV glucose administration and were deficient compared to young (656 ± 164 pmol/l, *p* < .001 when compared to basal; Figure 3).

**IVGTT and simultaneous iv GLP-1 (0.4 nmol/kg) administration.** — Serum glucose was not altered by GLP-1 during the IVGTT in the young animals, which resulted in identical K values (Figure 2, Table 1). Insulin responses were profoundly altered, however. Peak insulin at 2 min was 2,137 ± 286 pmol/l (*p* = .04 when compared to basal). GLP-1 restored the acute insulin response to glucose in the old animals (*p* = .006, one-way ANOVA).

<p>| Table 1. Elimination Rate Constant (k) During an Intravenous Glucose Tolerance Test (0.5 g/kg, glucose/body weight) With or Without GLP-1 in Wistar Rats |
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<table>
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<th>3-Month (n = 6)</th>
<th>22-Month (n = 6)</th>
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<tr>
<td>IVGTT</td>
<td>6.6 ± 0.8</td>
<td>3.8 ± 0.8</td>
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<td>IVGTT + GLP-1 (0.4 nmol/kg)</td>
<td>6.6 ± 0.7</td>
<td>4.8 ± 0.6*</td>
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*IVGTT in 22-month-old rats with vs without GLP-1.

*p* = .04 by Student’s *t*-test.

In the old animals AUCo-t for glucose was significantly decreased in the presence of GLP-1 (5,681 ± 130 vs 6,272 ± 251 mg/dl, *p* = .0164 by one-way ANOVA; Figure 2), although the peak glucose level was not altered. The K value during control IVGTT was 3.8 ± 0.8, and by comparison, there was a significant improvement in the K value with GLP-1 treatment (4.8 ± 0.6, *p* = .04, Table 1). The most profound change was that GLP-1 restored the acute insulin response during the IVGTT (1,659 ± 284 vs 384 ±
122 pmol/l, p = .002) at 2 min and significantly increased the AUC_{0-32} for insulin (16,160 ± 3,232 vs 7,973 ± 2,096 pmol/l, p = .012, Figure 3).

Two-way analysis of variance of serum insulin levels after simultaneous GLP-1 and IVGTT showed no significant difference between young and old rats. This finding further shows that age does not impair the ability of beta cells to respond to GLP-1. On the contrary, serum glucose analyzed under the same conditions showed that age had an effect on the ability of GLP-1 to control glycemia (p < .0001), as determined by two-way ANOVA.

DISCUSSION

Old rats appear to respond equally as well as young ones to intravenous GLP-1 in the fasted state. They achieved the same maximum insulin secretory response at the same time point after every bolus of GLP-1. A maximum insulin response was also seen with the same GLP-1 concentration in both ages (0.4 mmol/kg). Pancreatic beta cells therefore retain responsivity to the insulinoisotropic effects of GLP-1 with increasing age in Wistar rats. Normal human subjects also increase insulin release in response to GLP-1 infusion during euglycemia. Therefore, insulin response to GLP-1 is similar in both rats and humans in not requiring elevated glucose levels (i.e., not glucose-dependent) for insulin release (Elahi et al., 1994).

There were clear differences in the insulin responses during the IVGTT between young and old control animals. The acute insulin response was slower in old animals and did not reach as high a level. It has been shown that pancreatic beta cells are rendered glucose-competent by the insulinoisotropic effects of GLP-1 (Holz et al., 1993). Using the reverse hemolytic plaque assay, in an insulinoma cell line, we showed that GLP-1 causes more beta cells to respond to glucose when it is present in conjunction with glucose than do beta cells that see glucose alone (Montrose-Rafizadeh et al., 1994). We have also shown that fewer beta cells from old animals respond to glucose, especially in the acute phase of insulin release (Perfetti et al., 1995). Therefore GLP-1 in vivo is almost certainly inducing nonglucose responsive cells into a responsive mode to the extent that the older animals have restoration of their acute phase insulin release in response to glucose. As a result, glucose is eliminated more quickly during the IVGTT.

Acute phase insulin release is thought to have a critical physiological significance for glucose metabolism. In Type II diabetes its loss is an early feature (Palmer and Ensinck, 1975). Somatostatin infusions into nondiabetic subjects experimentally can inhibit the acute phase insulin release and cause an impairment of glucose tolerance and blunting of the thermic effect of glucose (Calles-Escandon and Robbins, 1987). The initial sharp rise in insulin causes activation of insulin receptors and, as a result, facilitative glucose transporters are shuttled to the plasma membrane to initiate insulin-mediated glucose uptake (Czech et al., 1992). The insulin receptor is activated in a concentration-dependent manner by insulin. Thus, the greater the insulin response to glucose during the acute phase, the greater should be the activation of insulin receptors. It is therefore not surprising that glucose tolerance was improved when we restored acute phase insulin release in our old rats.

It has been suggested that the loss of the acute phase insulin release is a consequence of reduced beta cell mass (Luft, 1984; Srikanta et al., 1984). Its loss is seen in Type I diabetes, where beta cell loss definitively occurs and where it is associated with the development of islet cell antibodies (Srikanta et al., 1984). However, if it were solely due to loss of beta cell mass we would be unable to significantly restore it with GLP-1. In any case, we have already demonstrated that beta cell mass is not reduced in aging (Perfetti et al., 1995). Rather, the loss of acute phase insulin release is a functional defect in which fewer beta cells are responsive. The effect of GLP-1 in restoring acute phase insulin response strengthens our previous demonstration of the effects of aging and GLP-1 treatment in vitro (Montrose-Rafizadeh et al., 1994; Perfetti et al., 1995).

However, despite restoration of the acute insulin response to glucose and despite achieving somewhat similar peak insulin levels in the presence of GLP-1 during the IVGTT, the elimination of glucose was still not fully normalized in the old animals. Insulin resistance therefore plays a role in the elevated levels of glucose seen after the IVGTT in old animals. Insulin resistance has already been shown to occur in the aging Wistar rat by other researchers in the field of aging and glucose intolerance (Nadiv et al., 1992). As already stated, GLP-1 did not alter the rate of elimination of glucose during the IVGTT in young animals. Young animals have therefore already achieved maximum glucose uptake in insulin-sensitive tissues with the insulin release attained by glucose during the IVGTT alone. Old animals, in contrast, having insulin resistance, are able to overcome some, but not all, of the resistance due to the increase in insulin levels attained by GLP-1 during the IVGTT. This results in an increase in glucose elimination and, therefore, decreased glucose levels.

In conclusion, old rats retain their ability to respond to the insulinoisotropic action of GLP-1 similar to young animals. The glucose responsivity of the pancreas is, however, severely altered with aging. Acute phase insulin release is lost in response to an IVGTT. This is similar to the pancreatic lesion described in Type II diabetes in humans. GLP-1 is capable of restoring glucose responsivity to the aged pancreas and of restoring acute phase insulin release. As a consequence, glucose tolerance is improved. Our data underscore the importance of GLP-1 as a treatment for Type II diabetes.

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