GLUCAGON-LIKE PEPTIDE 1 [GLP-1-(7—36) amide (GLP-1)] is a gut hormone that is released from the intestinal L cells during a meal and that, as an incretin factor, stimulates insulin secretion (3, 27, 28, 34, 40, 44). The peptide reduces circulating levels of glucose both in normal subjects and in subjects with type II diabetes (21, 22, 35, 42, 43, 48, 58). Therefore, GLP-1 is considered a potential agent for the treatment of diabetes (3, 24, 41).

Although GLP-1 thus stimulates insulin secretion, some studies have suggested that the peptide lowers circulating glucose after intravenous glucose also by actions independent from this effect. Thus GLP-1 has been demonstrated to increase insulin-dependent glucose disposal, i.e., to exert peripheral insulin-like effects (2, 3, 23, 39, 44, 47, 54, 56), although studies also refuting such effects or suggesting them to be of only minor importance have been published (6, 17, 25, 30, 35, 45, 55). Moreover, GLP-1 has in one study (10) been shown to stimulate the so-called glucose effectiveness (SG) in humans, as calculated by the minimal model approach. This action is hypothetically caused by stimulation of the insulin-independent glucose uptake, which is executed by glucose per se augmenting glucose uptake in the brain and in skeletal muscle and suppressing glucose output from the liver during hyperglycemia (1, 9). However, such an action of GLP-1 has not been demonstrated in other studies. Therefore, the mechanism of the glucose-reducing effect of GLP-1 might be complex, and the relative contribution of the potential mechanisms has still not been established.

The aim of the present study was therefore to examine in more detail the integrated dose-related net influence of GLP-1 on the processes affecting glucose homeostasis. In particular, we studied whether GLP-1 affects glucose disposal not only by potentiating glucose-stimulated insulin secretion but also by affecting the insulin-dependent and the insulin-independent (glucose dependent) glucose elimination, which is not established. Establishing these mechanisms is not only of interest for the understanding of the physiology of GLP-1 but is also required for the development of GLP-1 as a treatment of diabetes (3, 24, 41). To accomplish this goal, we used the minimal model analysis of glucose disappearance for glucose and insulin data analysis after an intravenous glucose tolerance test in the mouse, in conjunction with administration of GLP-1 over a wide dose range. This method allows estimation of acute insulin secretion, total insulin sensitivity (the ability of insulin to enhance glucose disappearance and to inhibit glucose production), $S_G$ (glucose disappearance per se at basal insulin without any change in insulin), and the glucose elimination rate ($K_G$) (7, 14). The method was initially developed for studies in humans (7, 14). Recently, we exploited this method also in vivo in mice by taking seven samples over 50 min (13). The use of seven samples for the minimal model analysis in mice was found to be sufficient because the model is based on the change in only two variables (insulin and glucose), because levels of insulin and glucose have returned to baseline levels in the last sample taken (at 50 min), and because the time points selected for the sampling accurately reflect the pattern of insulin and glucose changes after an intravenous glucose administration in mice (13). To examine by what extent increased insulin levels contribute to the effects of GLP-1, influences of raised plasma insulin levels matching the levels seen after GLP-1 were studied by administration of exogenous insulin. Finally, the influence of the GLP-1 receptor antagonist exendin-3-(9—39) was also studied. Exendin-3-(9—39) is a peptide isolated from the venom of Heloderma horridum and described as an antagonist for the GLP-1 receptors (12). The peptide has previously been shown...
to antagonize GLP-1-induced actions on insulin secretion in vitro as well as in vivo in humans, baboons, and rats (11, 18, 33, 52).

MATERIALS AND METHODS

Animals. A total of 301 nonfasted NMRI mice (Bomholtgaard Breeding and Research Center, Ry, Denmark), weighing 20–25 g, were used throughout the study. The animals were fed a standard pellet diet and tap water ad libitum.

Intravenous glucose tolerance test. The mice were anesthetized with an intraperitoneal injection of midazolam (0.4 mg/mouse; Dormicum, Hoffman-La Roche, Basel, Switzerland) and a combination of fentanyl (0.02 mg/mouse; Hypnorm, Janssen, Beersel, Belgium). Thereafter, a blood sample was taken from the retrobulbar, intraorbital, capillary plexus in heparinized tubes, and thereafter α-glucose (1 g/kg; British Drug Houses, Poole, UK) was rapidly injected intravenously in a tail vein either alone or together with synthetic GLP-1 at various dose levels (Peninsula Laboratories Europe, Merseyside, UK). In two other experimental series, human insulin (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) or synthetic exendin-3–(9–39) (Peninsula) was injected intravenously; insulin was administered together with glucose, whereas exendin-3–(9–39) was administered at 5 min before glucose or glucose + GLP-1. The volume load was 10 µl/g body weight. New blood samples were taken after 1, 5, 10, 20, 30, and 50 min. The samples were taken in heparinized tubes. After immediate centrifugation, plasma was separated and stored at −20°C or until analysis.

Analysis. Plasma insulin was determined radioimmunologically with the use of a guinea pig anti-rat insulin antibody and 125I-labeled human insulin as tracer and rat insulin as standard (Linco Research, St. Charles, MO). Free and bound radioactivity was separated by use of an anti-IgG (goat anti-guinea pig) antibody (Linco). The sensitivity of the assay is 12 pmol/l, and the coefficient of variation is <3%. Plasma glucose was determined with the glucose oxidase method.

Data analysis. Insulin and glucose data from the seven-sample intravenous glucose tolerance test were analyzed with the minimal model technique (1, 8). The model assumes a first-order nonlinear insulin controlled kinetic and accounts for the effect of insulin and glucose itself on glucose disappearance after exogenous glucose injection. It provides the parameter S_I, which is defined as the ability of insulin to enhance glucose disappearance per se (7), and the parameter S_G, representing glucose disappearance rate. The calculations of the parameters use the whole data set from 0 to the end of the experiment (9). Acute insulin secretion (AIR) was calculated as the mean of suprabasal 1- and 5-min insulin levels, and the areas under the curve (AUC) for insulin (AUC_insulin) and glucose (AUC_glucose) were assessed with the trapezoidal rule of suprabasal values. We also calculated a unitless index called global disposition index (GDI) by multiplying S_I times AUC_insulin. Finally, the K_G after the glucose injection was calculated with the half-time for the minutes 1-20 after glucose injection after logarithmic transformation of the individual plasma glucose values.

Statistics. Means ± SE are shown. Statistical analyses were performed with the SPSS for Windows system. Statistical comparisons between groups were performed with ANOVA followed by Bonferroni post hoc analysis. Pearson’s product moment correlation was used to estimate linear relationships. Hyperbolic regressions were calculated with the Sigma-plot 7.0 for Windows program (SPSS).

RESULTS

Effects of GLP-1 (10 nmol/kg) on insulin secretion and glucose disappearance. Figure 1 shows that the intravenous injection of glucose (1 g/kg; n = 24) rapidly increased plasma insulin levels with a peak level of 1,551 ± 239 pmol/l after 1 min (P < 0.001). Plasma
insulin levels thereafter returned to baseline levels within the 50-min study period. Concomitant administration of GLP-1 at 10 nmol/kg (n = 24) markedly potentiated the glucose-induced increase in plasma insulin levels, which peaked at the level of 7,444 ± 527 pmol/l after 5 min. Plasma insulin levels were significantly higher after administration of GLP-1 together with glucose than after administration of glucose alone at minutes 1, 5, and 10 (all P < 0.001) and at 20 min (P = 0.003) after administration. At 50 min, plasma insulin levels were slightly lower in animals given GLP-1 than in controls (P = 0.022). Also, plasma glucose levels peaked at 1 min after glucose administration, and thereafter plasma glucose levels returned to baseline levels within the 50-min study period. GLP-1 markedly enhanced the glucose elimination, and, accordingly, plasma glucose levels were lower after GLP-1-glucose than after glucose alone at 10, 20, 30, and 50 min (all P < 0.001). Insets in Fig. 1 show the parameters calculated from the seven-sample minimal model in this experimental series. The potentiated insulin secretion due to GLP-1 is evident by increased AIR and AUCinsulin (P < 0.001), whereas the increased glucose tolerance is evident by reduced AUCglucose and increased KG (P < 0.001). It is also seen that S1 was significantly reduced by GLP-1 (P < 0.001), whereas S2 was not significantly altered by GLP-1 (P = 0.126). Insulin secretion was stimulated more potently than insulin sensitivity was reduced by GLP-1. Therefore, the GDI was increased by GLP-1 because it was 18.0 ± 1.3 after GLP-1 vs. 12.4 ± 1.2 in controls (P = 0.004).

Figure 2 shows the negative correlation between AUCglucose and AIR in animals given GLP-1 at 10 nmol/kg (n = 24) and controls run in the same experiments (n = 24). It is seen that these two parameters displayed a hyperbolic relation with a high correlation coefficient (r = −0.89, P < 0.001).

Dose-response relationship between dose of GLP-1 and insulin secretion. Figure 3 shows that insulin secretion, as judged both by AIR and AUCinsulin, increased dose dependently in response to administration of GLP-1. The two processes showed different sensitivity to GLP-1, because the threshold dose level for stimulating AIR was 0.1 nmol/kg and the corresponding level for increasing AUCinsulin was 1 nmol/kg. Furthermore, the half-maximal dose of GLP-1 for increasing AIR was −1 nmol/kg, whereas it was −3 nmol/kg for AUCinsulin.

Dose-response relationship between dose of GLP-1 and glucose tolerance. Figure 4 shows that GLP-1 dose dependently increased glucose tolerance. This was evident by reduction of AUCglucose and increase of KG after GLP-1. These two processes showed similar sensitivity to GLP-1, because the GLP-1 threshold dose (−0.1 nmol/kg) and half-maximal values (−3 nmol/kg) were the same.

Dose-response relationship between dose of GLP-1 and SI and S2. Figure 5 shows that after administration of GLP-1, S1 was reduced. The threshold dose level of GLP-1 eliciting this effect was −0.3 nmol/kg and...
eliciting half-maximal effect of ~1 nmol/kg. In contrast, SG was not affected by GLP-1 at any of the dose levels tested.

Comparison of GLP-1 and insulin. The results thus show that, besides stimulating insulin secretion, GLP-1 also exerted profound influences on glucose elimination (stimulation) and SI (inhibition). To establish whether these actions are dependent on the marked increase in plasma insulin or are induced by an insulin-independent action of GLP-1, insulin was exogenously administered at four different dose levels together with glucose to match the peripheral insulin levels achieved after administration of GLP-1 (Fig. 6). To match the plasma insulin levels seen after GLP-1 administration, two approaches had to be undertaken, because the time pattern of plasma insulin after administration of insulin was different from that after administration of GLP-1. A first approach was to match the rapid increase in plasma insulin after GLP-1, i.e., the AIR. This was executed by administration of insulin at 0.5 U/kg. However, the duration of the process of insulin administration is shorter than the process of endogenous insulin secretion in response to GLP-1 administration. This results in a lower AUC<sub>insulin</sub> after insulin administration at this dose level than after GLP-1 administration. In contrast, administration of insulin at 1 U/kg matched the AUC<sub>insulin</sub> seen after GLP-1, albeit at a higher AIR. The results show that insulin reduced SI and increased glucose tolerance, as judged by reduced AUC<sub>glucose</sub> and increased K<sub>G</sub> (Table 1). When their influence on SI was compared, the inhibitory effects of GLP-1, insulin at 0.5 U/kg, and insulin at 1 U/kg were not significantly different. Similarly, the reduction of AUC<sub>glucose</sub> and the increase in K<sub>G</sub> did not differ between the groups. Therefore, the results show that exogenous administration of insulin matching the plasma insulin seen after GLP-1 administration reproduces the same actions as GLP-1, indicating that the influences of GLP-1 are mediated mainly by the increased plasma insulin levels.
Effect of exendin-3-(9—39) on parameters calculated from the minimal model. To study whether the GLP-1 receptor antagonist exendin-3-(9—39) also antagonizes the stimulatory action of GLP-1 on insulin secretion and glucose tolerance in mice, we administered the compound (30 nmol/kg) 5 min before the administration of glucose with or without addition of GLP-1 at 10 nmol/kg. Figure 7 shows that exendin markedly reduced the insulinotropic action of GLP-1 and attenuated the increase in glucose tolerance. Thus plasma insulin levels at minutes 5 ($P < 0.012$), 10 ($P < 0.001$), and 20 ($P < 0.014$) after the administration of glucose 1 GLP-1 were lower in animals also given exendin-3-(9—39), and, correspondingly, the plasma glucose levels at minutes 10, 20, 30, and 50 were higher (all $P < 0.001$). In contrast, exendin-3-(9—39) did not significantly affect insulin secretion or glucose tolerance after intravenous glucose challenge.

Table 1. Parameters calculated from seven-sample minimal model in mice iv injected with glucose together with GLP-1 or insulin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>GLP-1, 10 nmol/kg</th>
<th>Insulin, 0.1 U/kg</th>
<th>Insulin, 0.25 U/kg</th>
<th>Insulin, 0.5 U/kg</th>
<th>Insulin, 1 U/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>24</td>
<td>10</td>
<td>14</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>Air, pmol/l</td>
<td>$6,061 \pm 413$</td>
<td>$1,286 \pm 166^*$</td>
<td>$2,766 \pm 402^*$</td>
<td>$5,363 \pm 381$</td>
<td>$10,777 \pm 657^*$</td>
</tr>
<tr>
<td>$AUC_{insulin}$, nmol/l in 50 min</td>
<td>$105 \pm 9.0$</td>
<td>$22.2 \pm 2.4^*$</td>
<td>$31.2 \pm 3.0^*$</td>
<td>$47.4 \pm 3.0^*$</td>
<td>$97.2 \pm 7.2$</td>
</tr>
<tr>
<td>$AUC_{glucose}$, mmol/l in 50 min</td>
<td>$487 \pm 16.8$</td>
<td>$784 \pm 33.6^*$</td>
<td>$616 \pm 22.4^*$</td>
<td>$468 \pm 16.8$</td>
<td>$504 \pm 11.2$</td>
</tr>
<tr>
<td>$K_e$, %/min</td>
<td>$6.0 \pm 0.3$</td>
<td>$2.0 \pm 0.2^*$</td>
<td>$5.0 \pm 0.5$</td>
<td>$5.3 \pm 0.3$</td>
<td>$4.6 \pm 0.2$</td>
</tr>
<tr>
<td>$S_s$, 10^{-4} min^{-1}/pmol/l</td>
<td>$0.19 \pm 0.01$</td>
<td>$0.70 \pm 0.15^*$</td>
<td>$0.63 \pm 0.08^*$</td>
<td>$0.40 \pm 0.04$</td>
<td>$0.16 \pm 0.02$</td>
</tr>
<tr>
<td>$S_g$, min^{-1}</td>
<td>$0.047 \pm 0.003$</td>
<td>$0.074 \pm 0.018$</td>
<td>$0.071 \pm 0.004$</td>
<td>$0.067 \pm 0.006$</td>
<td>$0.073 \pm 0.072$</td>
</tr>
<tr>
<td>GDI</td>
<td>$18.0 \pm 1.3$</td>
<td>$17.1 \pm 5.9$</td>
<td>$17.4 \pm 2.6$</td>
<td>$17.7 \pm 1.3$</td>
<td>$15.1 \pm 1.1$</td>
</tr>
</tbody>
</table>

Values are means ± SE. Statistical evaluation by data with ANOVA with Bonferroni post hoc analysis revealed significant difference vs. glucagon-like peptide 1 (GLP-1) group of $^*P < 0.001$, $^†P < 0.01$ and $^‡P < 0.05$. Air, acute insulin secretion; $AUC_{insulin}$ and $AUC_{glucose}$, areas under insulin and glucose curves, respectively; $K_e$, rate of elimination; $S_s$, sensitivity index; $S_g$, glucose effectiveness; GDI, global disposition index.
DISCUSSION

In this study, we confirm that GLP-1 potentiates glucose-stimulated insulin secretion in vivo (3, 4, 16, 21, 22, 27, 34, 35, 42, 43, 48, 58) and we also show that this action of GLP-1 is accompanied by a potent increase in glucose tolerance. This enforces the consideration of the future use of the peptide in the treatment of diabetes (3, 24, 41). What is still controversial is whether the potentiation by GLP-1 of glucose tolerance is solely explained by its insulinotropic action or whether other mechanisms, such as stimulation of insulin-dependent and insulin-independent, glucose-dependent glucose uptake, contribute. The main novelty of the present study is to solve these important issues, because it shows the combined effects of GLP-1 on the processes affecting glucose disposal and defines the dose-response relationship between GLP-1 and insulin secretion and glucose tolerance under carefully undertaken in vivo conditions. We used the minimal model approach in mice for analysis of glucose disappearance to explore several aspects of glucose tolerance, because the model provides important parameters characterizing insulin secretion as well as insulin and glucose action on glucose homeostasis (7, 8, 13, 14). The Sₐ, as determined by this technique might overestimate insulin sensitivity as determined by clamp techniques (50, 51). However, the relative measures derived are valid, because it has been demonstrated that regular and insulin-modified intravenous glucose tolerance tests (with different insulin levels) give the same Sₐ (46).

The results of the study show that the stimulation of glucose tolerance by GLP-1 is entirely explained by its insulinotropic action and does not involve any stimulation of insulin-dependent or insulin-independent peripheral glucose uptake. The most prominent action of GLP-1 on the parameters derived from the analysis was its potentiating action on glucose-stimulated insulin secretion. This effect of GLP-1 showed a high potency because it was evident already at the low dose of 0.1 nmol/kg and because the half-maximal effect was achieved at a dose level of ~0.1 nmol/kg. GLP-1 also stimulated insulin secretion by a high efficiency, because at the maximal dose level an almost 10-fold potentiation of glucose-stimulated insulin secretion was exerted by the peptide. This potent insulinotropic action of GLP-1 is well-known from previous reports both in vivo in humans and in animals (3, 4, 16, 21, 22, 26, 27, 34, 35, 42, 43, 48, 58) and in vitro in islets and perfused pancreas (15, 19, 28, 29, 40, 57). In humans, a dose-response study has revealed that a rapid subcutaneous administration of GLP-1 at 0.5 nmol/kg is the threshold dose for stimulating insulin secretion because no effect was observed at the lower dose of 0.15 nmol/kg (48). Furthermore, a study in patients with type II diabetes has revealed that repeated subcutaneous administrations of ~0.3 nmol/kg elicited insulin secretion (31). This shows a similar sensitivity for GLP-1 to augment insulin secretion in humans and in the mouse.

In previous studies in unanesthetized mice, we found that a dose level of GLP-1 of 1 nmol/kg was insufficient to stimulate insulin secretion unless circulating glucose was raised to levels >20 mmol/l (4, 16), whereas a dose of 0.1 nmol/kg was sufficient to stimulate insulin secretion in the present study in anesthetized mice. This shows that anesthetized animals are more sensitive in the detection of insulinotropic action of GLP-1, which might be explained by the circulating catecholamines, the levels of which are lower in anesthetized mice than in unanesthetized mice (13, 32) and the action of which inhibits insulin secretion (5).

The action of GLP-1 to potentiate glucose-stimulated insulin secretion is most likely exerted through a direct action on the islet B cells, considering that these cells are equipped with GLP-1 receptors (53) and that GLP-1 stimulates insulin secretion in vitro (15, 16, 19, 28, 29, 40, 57). This action is to a large degree explained by the enhancement of cAMP formation, although other mechanisms might contribute (3, 20). The insulinotropic action of GLP-1 is glucose dependent (3, 4, 16, 19, 24, 26, 29, 44, 48, 57). Thus a glucose level of ~3 mmol/l is required for an insulinotropic action of GLP-1. The rapid inhibition of GLP-1-induced insulin secretion after reduction in glucose was seen in the present study, in which the 50-min values of circulating insulin were significantly lower after administration of GLP-1 than in controls even though the levels had been markedly elevated before this time point. This is explained by the lower circulating glucose at 20 and 30 min after GLP-1 administration than in controls, after which the insulinotropic action of the peptide vanishes.

From the comparison of the two parameters for insulin secretion (AIR and AUC insulin), it is evident that AIR was more sensitive than AUC insulin in detecting the insulinotropic action of GLP-1. Thus, at a dose as low as 0.1 nmol/kg, GLP-1 significantly increased AIR, whereas a dose level of 3 nmol/kg was required for a robust potentiation of AUC insulin by GLP-1. This is because the action of GLP-1 is short after intravenous administration, due to the very short half-life of GLP-1, which has been shown to be only ~4 min (34). Accordingly, AIR, which determines the action of GLP-1 only during the first 5 min after administration, was more sensitive to detect an effect of GLP-1 than AUC insulin, which takes into account the entire 50-min study period. Also, the lowered insulin levels at 50 min after GLP-1 administration influence the calculation of AUC insulin but not AIR. At the higher doses of GLP-1, the potent insulinotropic action of GLP-1 is prolonged, causing a right shift of the temporal pattern of plasma insulin levels, resulting in a peak level not at 1 min but at 5 min after administration.

We also demonstrate that GLP-1 potently exaggerated glucose elimination, as judged both as a reduced AUC glucose and as an increased K G, the latter being threefold increased. Furthermore, the 30-min values of circulating glucose were lower after GLP-1 administration than in glucose-injected controls. The potency of GLP-1 to increase the glucose tolerance was similar to its potency to stimulate insulin secretion. Previously,
increased glucose tolerance after intravenous glucose injection has been demonstrated by GLP-1 in humans (10, 48). This increased glucose tolerance after GLP-1 administration might be explained by several factors. One obvious possibility is that GLP-1 accentuates glucose elimination through its insulinotropic action. However, other possibilities also exist. Thus GLP-1 could, in addition, augment insulin-stimulated glucose uptake. The possibility of such an action has been raised by results from in vitro studies that have suggested that GLP-1 enhances insulin-stimulated glucose uptake in adipocytes and skeletal muscle (39, 47, 56), although studies also showing no or only minor effects of GLP-1 on glucose metabolism in skeletal muscle have been published (17, 25). In the present study, we did not detect any stimulation by GLP-1 of insulin sensitivity. Instead, an important observation in our present study is that the minimal model analysis revealed that SI was reduced after administration of GLP-1. This is similar to what previously was observed after administration of the neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP; Ref. 13). This raises the issue whether GLP-1, as well as PACAP, inhibits insulin action under in vivo conditions. This could theoretically be due to indirect actions, for example, if catecholamines were released by these peptides and through activation of β-adrenoceptors would induce insulin resistance (cf. 36). An alternative suggestion, however, is that GLP-1 induces such a marked increase in plasma insulin that insulin reduces its own action by causing insulin resistance, as inferred from human studies showing that insulin infusion may induce insulin resistance (37, 38, 49). To examine this possibility, we examined the influence of a rapid intravenous administration of insulin on SI. The temporal pattern of plasma insulin levels after insulin administration was different from that after GLP-1 administration because the process of insulin administration is of a shorter duration than the process of endogenous insulin secretion after GLP-1 administration. In one series of experiments, we therefore matched the AUC insulin. In both series, we found that SI was markedly lowered by insulin. This therefore suggests that the marked and rapid increase in circulating insulin after GLP-1 administration results in a prompt reduction in SI. Consequently, the reduction in SI after GLP-1 seems mediated by insulin. In fact, we have no indication of any action of GLP-1 per se on insulin sensitivity. The failure of GLP-1 per se to affect insulin action is similar to previous reports in humans and rats that stated that the peptide does not alter the insulin sensitivity during a hyperinsulinemic euglycemic clamp or the glucose disposal during glucose turnover studies by means of tracer infusion techniques (6, 10, 30, 45, 55). The mechanism of the reduction in insulin sensitivity upon a prompt rise in circulating insulin is not known. One possibility might be that the insulin effect is markedly reduced by the very high level of insulin obtained under these experimental conditions, perhaps as a safeguard against hypoglycemia and possibly executed by down-regulation of insulin receptors. Another possibility would be that counterregulatory hormones are liberated due to the rapid fall in circulating glucose to prevent frank hypoglycemia, which would also be measured as a reduced SI. A third possibility is that the effect of insulin on glucose disposal is not linear within the concentration range used even without counterregulatory effects of insulin-induced insulin resistance. The present study cannot distinguish between these processes because the conclusion is based on the finding that glucose disappearance did not increase as much as insulin levels. Hence, the model used can only quantify the processes as a whole, and further studies are required to elucidate the detailed mechanisms. Therefore, the conclusion that can be drawn is that the marked increase in glucose elimination induced by GLP-1 cannot be explained by increased insulin action.

It is known that the elimination of glucose is not only regulated by insulin-dependent mechanisms, but also by insulin-independent actions, mainly glucose uptake independent from insulin in the brain and in skeletal muscle and suppression of glucose output from the liver during hyperglycemia (1, 9). The minimal model technique reveals the ability of glucose itself to increase glucose uptake through a dynamic insulin-independent action, SI (9). In a previous report (10) in healthy human subjects, it was shown that GLP-1 increases SI. However, in the present study in mice, this was not reproduced, because SI was not significantly altered by GLP-1 at any of the concentrations examined. Similarly, exogenous insulin did not affect SI. Therefore, this study provides evidence that the main mechanism explaining the increased glucose elimination after GLP-1 is the marked insulinotropic action, which increases glucose tolerance despite a concomitant reduction in insulin sensitivity. This conclusion is consistent with the comparison of effects of GLP-1 and insulin administration: by matching the resulting plasma insulin levels, a similar increase in glucose tolerance, as judged by AUC glucose or K G, was evident. Hence, the increased insulin levels seen after GLP-1 administration overcome the reduction in insulin sensitivity, which is also evident by the significant increase in GDI induced by GLP-1.

In this study, we also examined the GLP-1 receptor antagonist, exendin, which is a peptide isolated from the venom of Heloderma horridum (12). This peptide has previously been shown to be a potent and selective antagonist of GLP-1 on insulin secretion both in vitro (35) and in vivo in humans (52), baboons (11), and rats (33). We show that exendin-3-(9—39) inhibits both the stimulation of insulin secretion and glucose tolerance after GLP-1 in mice. Exendin-3-(9—39) was very potent in this respect, because when combined with a maximal dose level of GLP-1 (10 nmol/kg), a dose only three times higher was sufficient to totally antagonize the influence of GLP-1 on glucose tolerance. Therefore, exendin-3-(9—39) is a well-suited peptide for study of the physiology of GLP-1 in the mouse.
Based on this integrative study with the minimal model analysis of glucose and insulin data obtained from the seven-sample technique in the mouse to study influence of GLP-1 on insulin secretion, insulin sensitivity, and glucose disposal, we conclude 1) that GLP-1 potently stimulates insulin secretion and increases glucose tolerance without altering the SGR, 2) that exogenous insulin administration matching the plasma insulin levels achieved by GLP-1 administration reproduces the influences of GLP-1, 3) that the rapid increase in insulin after administration of GLP-1 or insulin results in lowering of insulin sensitivity, and 4) that exendin-3-(9–39) antagonizes these actions of GLP-1. The study is consistent with the view that the stimulatory effect of GLP-1 on glucose elimination is executed by raised insulin levels and that exendin-3-(9–39) is well suited for use in studies of the physiology of GLP-1.

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