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Effects of glucagon-like peptide-I on glucose turnover in rats

Van Dijk, Gertjan, Stefan Lindskog, Jens J. Holst, Anton B. Steffens, and Bo Ahren. Effects of glucagon-like peptide-I on glucose turnover in rats. Am. J. Physiol. 270 (Endocrinol. Metab. 33): E1015–E1021, 1996.—The influences of glucagon-like peptide-I-(7–36) amide (GLP-I, 15 pmol·kg⁻¹·min⁻¹) on glucose turnover were studied in freely moving Wistar rats. In fed rats, GLP-I reduced plasma glucose (from 7.3 ± 0.2 to 5.6 ± 0.3 mmol/l; P = 0.017), increased plasma insulin (from 20 ± 3 to 89 ± 11 μU/ml; P = 0.002), and reduced plasma glucagon (from 44 ± 1 to 35 ± 2 pg/ml; P = 0.009) and glucose appearance rate (Rₐ; from 3.9 ± 0.2 to 1.7 ± 0.7 μmol·min⁻¹·100 g⁻¹ after 30 min; P = 0.049) without affecting glucose disappearance rate (Rₐ). The glucose clearance rate (MCR) was increased (P = 0.048). In 48-h-fasted rats, GLP-I reduced plasma glucose (from 5.0 ± 0.2 to 4.4 ± 0.3 mmol/l; P = 0.035) and increased plasma insulin (from 4 ± 1 to 25 ± 10 μU/ml; P = 0.042) and plasma glucagon (from 43 ± 3 to 61 ± 7 pg/ml; P = 0.046). Rₐ and Rₐ were not significantly affected, although Rₐ was lower than Rₐ after 15–30 min (P = 0.005) and MCR was increased (P = 0.049). Thus GLP-I reduces Rₐ in fed rats and increases MCR in fed and fasted rats. The reduced Rₐ seems mediated by an increased insulin-to-glucagon ratio, the increased glucose clearance seems dependent on insulin and a peripheral effect of GLP-I.

insulin secretion; glucagon secretion; tritiated glucose; fasting.

THE TRUNCATED AMIDATED FORM of glucagon-like peptide-I (GLP-I) is processed from proglucagon in L cells in the distal portion of the gut and is released into the bloodstream during and after food intake (3, 6, 10, 22). GLP-I has been shown to stimulate insulin secretion in both humans and experimental animals (1, 5, 8, 9, 11, 12, 15, 17, 18, 20, 26) and is considered an important incretin factor (3, 6, 10, 22). The peptide also inhibits glucagon secretion (9, 12, 20) and reduces plasma glucose levels (4, 21). Because of this unique pattern of effects, the peptide has attracted great interest as a potential treatment modality in type 2 diabetes (12, 20).

The mechanism underlying the reduced circulating glucose levels by GLP-I has not been established. In overnight-fasted human subjects, infusion of GLP-I reduces hepatic glucose delivery, which most likely is a consequence of increased insulin and decreased glucagon secretion (16). In addition, GLP-I increases the metabolic clearance of glucose, presumably because of increased insulin levels (16). There is also evidence, however, that GLP-I reduces circulating glucose levels by a peripheral insulin independent action, because the peptide increased glucose utilization during a hyperglycemic hyperinsulinemic clamp in type 1 diabetic patients (12). Furthermore, GLP-I might also reduce the glucose levels by potentiating the insulin-independent glucose disposal, as has been shown in fasted healthy human subjects (5). This is in agreement with our previous observation that the peptide reduced circulating glucose levels in mice which had been given 2,5-anhydro-D-mannitol, a compound known to inhibit glycogenolysis, gluconeogenesis, and insulin secretion (4). Alternatively, the effects described above might be explained by an inhibitory action of GLP-I on hepatic glucose delivery through a potentiation of the previously described glucose-dependent inhibition of hepatic glucose delivery (19). The differing results indicate that the relative contribution to the glucose-lowering effect of GLP-I by reduction of hepatic glucose delivery vs. stimulation of peripheral glucose disposal is still not established. Neither is it known whether the mechanism of the glucose-lowering effect of GLP-I is different under fasting and fed conditions.

To study further the mechanism of the glucose-lowering effect of GLP-I, we examined the influence of the peptide on hepatic glucose delivery and peripheral glucose disposal by use of the glucose tracer technique in rats under both fed and fasting (48 h) conditions.

MATERIALS AND METHODS

Animals. Male Wistar rats, weighing 290–320 g at the beginning of the present experiment, were used. The animals were individually housed in Plexiglas cages (25 × 25 × 30 cm) at room temperature (20 ± 2°C) and had continuous access to food (Hope farm chow) and water unless otherwise stated. The rats were maintained on a 12:12-h light-dark cycle (0700–1900 light), and they were handled and weighed every day. Under halothane anesthesia and 3 wk before the experiments, all rats were provided with a nonocclusive catheter in the abdominal artery according to techniques described elsewhere (29). This catheter allows frequent arterial blood sampling in freely moving undisturbed rats. For venous infusion of tritiated glucose and/or GLP-I solutions, rats were also provided with a silicon catheter that was inserted in the heart via the right jugular vein (28). Before experiments, catheters were filled with polyvinylpyrrolidone (mol wt 2,500).

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Experimental procedures. All experiments were performed in the light period between 1100 and 1400 in the home cage of the animals. Food was removed from the cage 2 or 48 h before the start of the experiment. One hour before blood sampling, venous and arterial catheters were connected to polyethylene tubes (length 0.4 m, OD 1.25 mm, ID 0.75 mm) filled with citrated saline. Forty-five minutes before the first blood
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RESULTS

Fed rats. On the experimental day, the ad libitum-fed rats receiving the GLP-I solution (n = 5) and vehicle (n = 6) weighed 357 ± 8 and 353 ± 5 g, respectively. Table 1 shows the baseline plasma glucose, insulin, and glucagon levels as well as the Rₜ, Rₐ, and the clearance rate of glucose. Fig. 1 shows the plasma levels of glucose, insulin, and glucagon before and during infusion of GLP-I (n = 5) or vehicle (n = 6) in the fed rats. Intravenous infusion of GLP-I reduced the plasma glucose levels from 7.3 ± 0.2 to 5.6 ± 0.3 mmol/l at 30 min, i.e., by 1.7 ± 0.5 mmol/l (P = 0.017). Thereafter the glucose levels gradually returned toward the preinfusion levels. Furthermore, GLP-I markedly, although transiently, increased plasma insulin levels from 20 ± 3 to 89 ± 11 μU/ml after 15 min of infusion, i.e., by 69 ± 10 μU/ml (P = 0.002). After 15 min of infusion, the plasma insulin levels returned to baseline despite the ongoing GLP-I infusion. The plasma insulin levels did not drop below those in the controls, even though the plasma glucose levels were lower. Plasma glucagon levels were constantly reduced during the GLP-I infusion, being 44 ± 1 pg/ml before GLP-I infusion and 35 ± 2 pg/ml after 45 min of peptide infusion (P = 0.009). After 15 min of GLP-I infusion, the plasma insulin-to-plasma glucagon ratio was 2.5 ± 0.4 μU/pg, whereas the corresponding ratio in saline-infused controls was 0.39 ± 0.03 μU/pg (P = 0.009). In the vehicle-infused control rats, no significant changes were observed in plasma glucose, insulin, or glucagon levels.

Figure 2 shows the Rₜ and Rₐ of glucose during the infusion of GLP-I and vehicle in fed rats. Rₜ was significantly reduced during the first 30 min of GLP-I infusion, because it fell from 3.9 ± 0.2 μmol·min⁻¹·100 g⁻¹ before the start of GLP-I infusion to 1.7 ± 0.7 μmol·min⁻¹·100 g⁻¹ during 30 min of infusion (P = 0.049). After 30 min, Rₜ returned to control levels despite the ongoing infusion of GLP-I and the constantly reduced plasma glucose levels. In contrast, Rₐ was not significantly affected by GLP-I. It was 3.6 ± 0.2 μmol·min⁻¹·100 g⁻¹ before and 3.1 ± 0.2 μmol·min⁻¹·100 g⁻¹ after 30 min of infusion (not significant (NS)). At the same time, however, plasma glucose values were lowered by GLP-I (see Fig. 1). Hence the glucose clearance rate, i.e., glucose Rₜ-to-plasma glucose ratio,
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Thereafter, plasma glucose levels gradually returned to baseline values. Similarly to the fed rats, GLP-I induced a transient increase in plasma insulin levels in the fasted rats, from 4 ± 1 to 25 ± 10 μU/mL after 15 min of infusion, i.e., by 21 ± 8 μU/mL (P = 0.042). Thereafter, the insulin values returned to the low baseline levels despite the ongoing GLP-I infusion. As in the fed rats, plasma insulin levels never dropped below control values, yet plasma glucose levels were lower in GLP-I- than in vehicle-infused rats. In contrast to what was observed in the fed rats, plasma glucagon levels transiently increased during the GLP-I infusion in the 48 h fasted rats, from 43 ± 3 to 61 ± 7 pg/ml, i.e., by 18 ± 6 pg/ml after 30 min of infusion (P = 0.046). The peak glucagon values coincided with the nadir glucose value; thereafter glucagon levels returned to preinfusion values. After 15 min of GLP-I infusion, the plasma insulin-to-plasma glucagon ratio was 0.49 ± 0.18 μU/pg, whereas the corresponding ratio in vehicle-infused controls was 0.06 ± 0.02 μU/pg (P = 0.041). However, after 30 min of infusion, the plasma insulin-to-plasma glucagon ratio had returned to 0.11 ± 0.04 μU/pg in the GLP-I-infused animals vs. 0.06 ± 0.02 μU/pg in the controls (NS). In the vehicle-infused control rats, no significant changes were observed in plasma glucose, insulin, or glucagon levels.

Figure 4 shows the glucose Rₐ and Rₜ during infusion of GLP-I and vehicle in the 48-h-fasted rats. Neither the glucose Rₐ nor the glucose Rₜ was significantly affected by GLP-I. Rₚ was significantly lower than Rₜ at the interval of 15–30 min after start of GLP-I infusion (0.83 ± 0.15 vs. 1.63 ± 0.15 μmol·min⁻¹·100 g⁻¹; P –

was significantly increased by GLP I, because it was 0.56 ± 0.04 ml·min⁻¹·100 g⁻¹ after 30 min of peptide infusion compared with 0.43 ± 0.04 ml·min⁻¹·100 g⁻¹ after infusion of vehicle (P = 0.048). Infusion of the vehicle did not significantly alter Rₐ, Rₚ, or the clearance rate of glucose.

Fasted rats. On the experimental day, the 48-h-fasted rats receiving the GLP-I solution (n = 5) and vehicle (n = 5) weighed 320 ± 7 and 320 ± 4 g, respectively. During the fasting period, rats lost on average 49 ± 2 g of body weight. Table 1 shows the baseline plasma glucose, insulin, and glucagon levels as well as the Rₐ, the Rₚ, and the clearance rate of glucose in the 48-h-fast ed rats. Figure 3 shows the plasma glucose, insulin, and glucagon levels in the 48-h-fasted rats infused with GLP-I (n = 5) or vehicle (n = 5). As in the fed rats, the intravenous infusion of GLP-I reduced the plasma glucose levels also in the fasted rats, from 5.0 ± 0.2 mmol/l before the infusion to 4.4 ± 0.3 mmol/l after 30 min of infusion, i.e., by 0.6 ± 0.2 mmol/l (P = 0.035).
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Fig. 3. Plasma glucose (A), insulin (B), and glucagon concentrations (C) in rats fasted for 48 h and infused intravenously with GLP-I (n = 5; 15 pmol·kg⁻¹·min⁻¹; ●) or vehicle (n = 5; ○). Infusions are as described in Fig. 1. Values are means + SE.

0.005; Fig. 5), which was at the time point when plasma glucose values were lowered by GLP-I (see Fig. 3). The glucose clearance rate, i.e., glucose Rd-to-plasma glucose ratio, was significantly higher after 30 min of infusion with GLP-I, 0.38 ± 0.04 ml·min⁻¹·100 g⁻¹, than during infusion of vehicle, 0.26 ± 0.03 ml·min⁻¹·100 g⁻¹ (P = 0.049). Vehicle infusion did not significantly alter glucose Rd, glucose Rg, or the clearance of glucose in the fasted rats.

Plasma GLP-I levels. Table 1 and Fig. 6 show plasma GLP-I levels before and during infusion of GLP-I and vehicle in freely fed and 48-h-fasted rats. The baseline plasma GLP-I levels were significantly higher in fed rats (18.5 ± 1.7 pmol/l) than in 48-h-fasted rats (12.6 ± 1.3 pmol/l; P = 0.013). The plasma GLP-I levels in fed and fasted rats during GLP-I infusions were not significantly different.

DISCUSSION

In the present study, we found that GLP-I transiently reduces the R₄ of glucose by 31% in freely fed rats, without affecting the R₃ of glucose. This resulted in a transient reduction of plasma glucose levels by 1.7 mmol/l (Fig. 1). The clearance rate of glucose, i.e., the rate by which each circulating millimole of glucose is cleared, was increased during GLP-I infusion, because glucose R₄ remained unaffected whereas plasma glucose levels decreased. Furthermore, the study demonstrates that the peptide increases plasma insulin and reduces plasma glucagon in fed rats. We interpret these findings to indicate that GLP-I in fed rats markedly increases the insulin-to-glucagon ratio, which reduces hepatic glucose output. The insulinotropic action of GLP-I vanished after 30 min, and, as a result, the low glucose R₄ increased again toward control values. Thus, despite levels of plasma glucagon constantly remaining low, GLP-I only transiently influenced glucose R₄. At the same time, GLP-I increases the efficacy of peripheral glucose uptake, which might be an effect mediated by insulin or directly stimulated by GLP-I.

Our results confirm the previously reported study in overnight-fasted humans, in which GLP-I reduced the
GLP-I was low in these rats. During GLP-I infusion, the increase in the ratio of plasma insulin to plasma glucagon in fasted rats is probably due to the fact that glucose $R_\text{a}$ was not altered during GLP-I infusion. This would suggest a change in the $R_\text{a}-R_\text{d}$ balance, which would indicate a stimulated clearance of glucose. The fact that glucose $R_\text{a}$ was not altered during GLP-I infusion in fasted rats is probably due to the fact that the increase in the ratio of plasma insulin to plasma glucagon was low in these rats. During GLP-I infusion in fasted rats, plasma glucagon levels were not reduced. This was not expected, because GLP-I has previously been shown to inhibit glucagon secretion in humans (12) as well as in experimental animals, including rodents (9, 23). It is likely that the hypoglycemic action of GLP-I under the 48-h-fasting condition led to an increased activation of the sympathoadrenal system (13), which may have counteracted the direct inhibitory action of GLP-I on glucagon secretion. Taken together, the results of the present study indicate that the glucose-lowering effect of GLP-I in fasted rats is mediated by stimulated glucose clearance rate and that the reduced $R_\text{a}$, which is seen only under fed conditions, is not directly mediated by GLP-I. Rather, the reduced $R_\text{a}$ seems to be mediated by the increased insulin-to-glucagon ratio in plasma, which was markedly increased by GLP-I under fed conditions but only mildly increased during fasting conditions. It cannot be excluded, however, that GLP-I in addition to its effects via pancreatic hormone secretion also directly reduces $R_\text{a}$ and that this effect was simply counteracted by the rising plasma glucagon levels due to hypoglycemia under fasting conditions. Therefore, more direct studies of the effects on GLP-I on liver cell glucose metabolism are required.

Insulin secretion was clearly and markedly stimulated by GLP-I, although this effect was more pronounced under fed than under fasted conditions. This could not be attributed to differences in concentrations of GLP-I in the circulation, because the infusion of GLP-I led to similar levels of plasma GLP-I in fed and fasted rats. Thus, this observation may reinforce the idea that the insulinotropic action of GLP-I is glucose dependent (3, 6, 10, 21). In particular, a recent study in the mouse showed a more marked insulinotropic action of GLP-I under fed conditions than during fasting (1). The finding in the present study, that the increased plasma insulin levels returned to preinfusion values already during the ongoing infusion of the peptide, has previously been demonstrated in humans as well (21). This phenomenon might be explained by the gradual lowering of plasma glucose levels, which weakens the insulin secretory action of the peptide. However, because the plasma glucose levels, at least under fed conditions, were still above levels required for the insulinotropic action of GLP-I, other explanations are also possible. For example, it may be speculated that GLP-I has induced a desensitization on the B cells during its infusion, as has been suggested from studies on the effect of GLP-I on adenosine 3',5'-cyclic monophosphate production in cultured cell lines transfected to express the GLP-I receptor (31). Whether desensitization occurs of GLP-I receptors located on B cells in vivo remains to be studied.

A question that still remains to be solved is whether the effects on glucose turnover observed during GLP-I infusion, i.e., reduced $R_\text{a}$ in fed rats and increased glucose clearance rate in both fed and fasted animals, are solely mediated by the increased insulin-to-glucagon ratio or whether a direct action of GLP-I contributes to these phenomena. The increased glucose clear.
ance induced by GLP-I could be at least partially mediated by a direct action of the peptide, because it was of the same magnitude in fed and fasted conditions, yet the insulinotropic action of the peptide was weaker under fasted conditions. Furthermore, because glucose \( R_g \) was unaffected during GLP-I infusion, an insulin-independent action seems to contribute to the increased glucose clearance. However, the exact mechanism underlying the increased glucose clearance during GLP-I infusion remains to be established.

In conclusion, we have demonstrated that the mechanism of the plasma glucose-reducing effect of GLP-I in the rat is dependent on the nutritional status. Thus, in the freely fed state, the peptide reduces plasma glucose by reducing hepatic glucose delivery and increasing the clearance rate of glucose, whereas, under fasting conditions, the glucose-lowering effect of GLP-I is mediated mainly by increasing the glucose clearance rate. Furthermore, our study presents evidence that the reduced glucose clearance rate of glucose, whereas, under fasting conditions, the glucose-lowering effect of GLP-I is mediated mainly by increasing the glucose clearance rate. Because diabetes is accompanied by inappropriately elevated hepatic glucose production and reduced glucose clearance (7), the inhibition of hepatic glucose output and the stimulation of the peripheral glucose uptake by GLP-I in the rat is dependent on the nutritional status. Thus, in the freely fed state, the peptide reduces plasma glucose by reducing hepatic glucose delivery and increasing the clearance rate of glucose, whereas, under fasting conditions, the glucose-lowering effect of GLP-I is mediated mainly by increasing the glucose clearance rate. Because diabetes is accompanied by inappropriately elevated hepatic glucose production and reduced glucose clearance (7), the inhibition of hepatic glucose output and the stimulation of the peripheral glucose uptake by GLP-I, together with its effects to stimulate insulin secretion and inhibit glucagon secretion, reinforce the need for further studies to explore the use of this substance or its analogues in the treatment of diabetes.

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REFERENCES


