Glucagon-like peptide-1 improves insulin and proinsulin binding on RINm5F cells and human monocytes

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Received 18 August 1999; accepted in final form 8 February 2000

Glucagon-like peptide-1 improves insulin and proinsulin binding on RINm5F cells and human monocytes. Am J Physiol Endocrinol Metab 279: E88–E94, 2000.—Glucagon-like peptide-1(7–36) amide (GLP-1) is a potent incretin hormone secreted from distal gut. It stimulates basal and glucose-induced insulin secretion and proinsulin gene expression. The present study tested the hypothesis that GLP-1 may modulate insulin receptor binding. RINm5F rat insulinoma cells were incubated with GLP-1 (0.01–100 nM) for different periods (1 min-24 h). Insulin receptor binding was assessed by competitive ligand binding studies. In addition, we investigated the effect of GLP-1 on insulin receptor binding on monocytes isolated from type 1 and type 2 diabetes patients and healthy volunteers. In RINm5F cells, GLP-1 increased the capacity and affinity of insulin binding in a time- and concentration-dependent manner. The GLP-1 receptor agonist exendin-4 showed similar effects, whereas the receptor antagonist exendin-(9–39) amide inhibited the GLP-1-induced increase in insulin receptor binding. The GLP-1 effect was potentiated by the adenylyl cyclase activator forskolin and the stable cAMP analog Sp-5,6-dichloro-1-b-D-ribofuranosylbenzimidazole-3',5'-monophosphorothioate but was antagonized by the intracellular Ca2+ chelator 1,2-bis(2-amino-2-methyl-1-naphthoxy)ethane-N,N,N',N'-tetraacetic acid-AM. Glucagon, gastric inhibitory peptide (GIP), and GIP-(1–30) did not affect insulin binding. In isolated monocytes, 24 h incubation with 100 nM GLP-1 significantly (P < 0.05) increased the diminished number of high-capacity/low-affinity insulin binding sites per cell in type 1 diabetics (9,000 ± 3,200 vs. 18,500 ± 3,600) and in type 2 diabetics (15,700 ± 2,100 vs. 26,200 ± 4,200). Based on our previous experiments in IEC-6 cells and IM-9 lymphoblasts indicating that the low-affinity/high-capacity insulin binding sites may be specific for proinsulin, Jehe, PM, Fussgaenger RD, Angelus NK, Jungwirth RJ, Saile B, and Lutz MP. Am J Physiol Endocrinol Metab 276: E262–E268, 1999 and Jehle, PM, Lutz MP, and Fussgaenger RD. Diabetologia 39: 421–432, 1996), we further investigated the effect of GLP-1 on proinsulin binding in RINm5F cells and monocytes. In both cell types, GLP-1 induced a significant increase in proinsulin binding. We conclude that, in RINm5F cells and in isolated human monocytes, GLP-1 specifically increases the number of high-capacity insulin binding sites that may be functional proinsulin receptors.

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AS EARLY AS 1906, the treatment of diabetes mellitus with an acid extract of duodenal mucous membrane was proposed (33). This idea was further pursued in 1929 by Zunz and LaBarre (48), who coined the term “incretin” for a humoral factor from the intestinal tract that releases insulin or potentiates the glucose-induced insulin release. The ability of an oral glucose load to release more insulin than an intravenous glucose infusion, despite a similar increase in the blood glucose level, is due to the release of incretin hormones (10). In recent years, evidence has accumulated that glucagon-like peptide-1 (GLP-1) is an important incretin hormone (3, 8, 26, 35–38). GLP-1 is derived from the posttranslational processing of proglucagon [proglucagon-(78–107) amide; see Ref. 2] and is secreted from the distal parts of the jejunum, ileum, and colon in response to mixed meals (11, 26). Despite the physiological importance of the enteroinsular axis, disruption of GLP-1 is associated with only modest glucose intolerance in GLP-1 receptor−/− mice (44). These animals exhibit compensatory changes in the enteroinsular axis via increased glucose-dependent insulinotropic polypeptide (GIP) secretion and GIP action (40). Although GLP-1 is supposed to play an important role in the regulation of food intake (19), available data suggest that GLP-1 signaling may not be essential for the regulation of satiety or body weight (6).

In clinical studies in diabetics of both types, GLP-1 has been shown to considerably improve diabetes control (9, 18, 33). In contrast to other drugs for the treatment of type 2 diabetes, GLP-1 possesses a “glucagonostatic” effect that is of high therapeutic relevance (11, 34). The mechanisms responsible for the actions of GLP-1 in peripheral tissues, such as fat, liver, and muscle, remain unclear. In the present study, we tested the hypothesis that GLP-1 may specifically modulate insulin receptor binding, as suggested by our
preliminary findings in RINm5F insulinoma cells (23). The present study extended these experiments and investigated the effects of GLP-1 agonistic and antagonistic peptides and structurally related gastrointestinal hormones. We further addressed the question whether GLP-1 may also modulate insulin receptors in other cell types and determined insulin receptor binding in monocytes isolated from healthy subjects and diabetic patients. Finally, additional experiments are provided, showing that GLP-1 increases the binding of proinsulin but not insulin-like growth factor I (IGF-I).

MATERIALS AND METHODS

Reagents. GLP-1-(7–36) amide, GLP-1-(7–37), exendin-4, exendin-(9–39) amide, GIP-(1–42), truncated porcine GIP-(1–30), forskolin, and human recombinant IGF-I were obtained from Saxion (Hannover, Germany). Human biosynthetic insulin, proinsulin, and glucagon were from Lilly (Indianapolis, IN), Sp-5,6-dichloro-1-β-d-ribofuranosyl-benzimidazole-3',5'-monophosphorothioate (cBiMPS) was from Biolog (Bremen, Germany), and 1,2-bis(0-aminophenoxy)ethane-N,N,N',N'-tetraacetic acid (BAPTA)-AM was from Calbiochem (Bad Soden-Taunus, Germany). Human biosynthetic [125I]Tyr-A14-insulin (specific activity 360 μCi/μg) was labeled and HPLC was purified by A. Liebe (Hoechst, Frankfurt, Germany). Human recombinant [125I]Tyr-A14 proinsulin (specific activity 233 μCi/μg) was labeled by the lactoperoxidase method and subsequently purified by HPLC, as previously described (24). [125I]IGF-I (specific activity 250 μCi/μg) was purchased from Amersham (Braunschweig, Germany). [125I]GBP-1-(7–36) amide was labeled using the chloramine T method and was purified by HPLC as described (17). The binding assay buffer consisted of 50 mmol/l HEPES, 10 mmol/l dextrose, 15 mmol/l sodium acetate, 5 mmol/l KCl, 120 mmol/l NaCl, 1.2 mmol/l MgSO4, 10 mmol/l CaCl2, and 0.1% BSA, pH 7.8 adjusted with NaOH. All chemicals were purchased from Merck (Darmstadt, Germany). Forskolin and cBiMPS were solved in DMSO and then diluted in HEPES assay buffer. All other reagents were solved in HEPES assay buffer.

RINm5F cell culture. RINm5F cells were kindly provided by H. P. T. Ammon (Tubingen, Germany). This established insulinoma cell line expresses insulin and GLP-1 receptors (14, 16). Cells were grown in RPMI 1640 (Biochrom, Berlin, Germany) and were supplemented with 25 mM HEPES, 120 mmol/l NaCl, 1.2 mmol/l MgSO4, 10 mmol/l CaCl2, and 0.1% BSA, pH 7.8 adjusted with NaOH. All chemicals were purchased from Merck (Darmstadt, Germany). Forskolin and cBiMPS were solved in DMSO and then diluted in HEPES assay buffer. All other reagents were solved in HEPES assay buffer.

Isolation of human monocytes. After informed consent from healthy nondiabetic volunteers (n = 1/3 [females (F)/males (M)]; age 28.0 ± 4.1 (SD) yr; body mass index 22.9 ± 1.5 kg/m²), type 1 diabetes patients (n = 1/3 (F/M); age 36.0 ± 4.0 yr; body mass index 25.6 ± 2.3; duration of diabetes mellitus 10.3 ± 5.6 yr; fasting blood glucose 9.8 ± 1.4 mmol/l; HbA1c, 9.0 ± 3.1%), and type 2 diabetes patients (n = 2/2 (F/M); age 56.8 ± 5.6 yr; body mass index 30.6 ± 3.2; duration of diabetes mellitus 11.8 ± 6.9 yr; fasting blood glucose 13.4 ± 1.1 mmol/l; HbA1c, 11.3 ± 2.6%), 40 ml venous blood were collected. Mononuclear cells were isolated by Ficol-Hypaque density gradient sedimentation (Sigma, Deisenhofen, Germany), as previously described (21). The mononuclear cell layer was removed and diluted in HEPES assay buffer to a final concentration of 10⁷ mononuclear cells/ml.

Viability as assessed by trypan blue exclusion was always >95%.

Receptor binding studies. Freshly isolated monocytes were incubated for 24 h with indicated concentrations of GLP-1 in binding assay buffer at 37°C. The cell suspension was washed two times and adjusted to a cell number of 10⁶ cells/ml in binding assay buffer. The binding assays with RINm5F cells were performed with confluent cell monolayers grown to a density of 1.5 × 10⁶/well. Both cell types were incubated in assay buffer and solvent with GLP-1 or other agents or solvent alone for the indicated time periods. After removing the incubation buffer and washing the cells two times, specific binding of insulin, proinsulin, IGF-I, and GLP-1 was determined from competitive binding studies as previously described (21). Briefly, cells were incubated in assay buffer with 10 pM of [125I]-labeled peptides and various concentrations (10 pM-1 μM) of unlabeled homologous peptides for 2 h at 15°C. Cell bound and free intact activities were measured in an automatic gamma counter with 70% efficiency. Degradation of labeled peptides was determined by measuring the ability of intact tracer peptides to precipitate in ice-cold 5% TCA. The percentage of degraded tracer was determined after centrifugation (2,000 g force, 10 min) from the increase in TCA-soluble radioactivity over that observed in control wells containing buffer but no cells. [125I]-labeled insulin, proinsulin, and IGF-I were >98% precipitable before and >90% precipitable at the end of each experiment. [125I]-GLP-1 was >97% precipitable before and >85% precipitable at the end of each experiment. Specific [125I]peptide binding was determined by subtracting the amount of radioactivity bound nonspecifically in the presence of 1 μM unlabeled homologous peptide. All binding data were corrected for nonspecific binding and tracer degradation. Peptide binding capacity (% specific tracer binding) and affinity (IC50 values indicating half-maximal tracer displacement) were calculated from computer-assisted competition-inhibition curves. The number of binding sites per cell and dissociation constant (Kd) values of binding affinity were estimated by computer-assisted Scatchard analysis (21, 24, 42). Data analysis. If not otherwise indicated, data are expressed as mean values ± SE of 3–17 independent experiments using separate sets of cells. Unpaired Student’s t-test was used for statistical comparisons. A P value <0.05 was considered statistically significant.

RESULTS

Binding of [125I]-GLP-1-(7–36) amide to RINm5F cells and monocytes. RINm5F cells displayed specific binding of [125I]-GLP-1-(7–36) amide (4.6%/10⁶ cells; n = 3) that was competed by unlabeled GLP-1 with high affinity (IC50: 0.2 nM; Kd: 0.3 nM). Using the same tracer in freshly isolated monocytes of two healthy subjects, only weak binding of [125I]-GLP-1-(7–36) amide could be measured (0.44 ± 0.11%/10⁶ cells) without out displacement by unlabeled GLP-1 given from 10 pM to 1 μM. Increasing the number of monocytes up to 4 × 10⁷ cells or performing the binding studies at 4°C did not change the negative results.

Effect of GLP-1 and related peptides on insulin binding in RINm5F cells. As shown in Fig. 1, GLP-1-(7–36) amide induced a time-dependent increase of specific insulin binding with significant effects already after 5 min. A twofold higher insulin binding was observed after 24 h incubation without further increases by
longer incubation periods (up to 72 h). GLP-1 yielded a dose-dependent stimulation of insulin binding, with half-maximal effects at 0.1 nM given for 24 h (Fig. 2).

We next investigated whether structurally or effect-related hormones may also modulate insulin receptor binding. RINm5F cells were incubated for 2 h with 1 nM of GLP-1 alone or in combination with the GLP-1 receptor agonist exendin-4 or the GLP-1 receptor antagonist exendin-(9–39) amide (8, 17). GLP-1-(7–36) amide alone increased insulin binding from 100% (control) to 142 ± 6.3% (n = 17). Similar effects were observed with GLP-(7–37), which increased insulin binding to 141.7 ± 18.4% (n = 3). Incubation with exendin-4 (1 nM) alone stimulated insulin binding from 100% (control) to 161 ± 6% (P < 0.001; n = 6). The GLP-1 antagonist exendin-(9–39) amide alone showed no significant effects (116 ± 8%; n = 6). The GLP-1-induced increase in insulin binding (150 ± 10%) could be effectively blocked by 100 nM of exendin-(9–39) amide (116 ± 9%, P < 0.05 vs. GLP-1 alone; n = 6). As shown in Fig. 3, the dose-dependent increase in insulin binding observed with GLP-1-(7–36) amide was not seen with glucagon, GIP, or GIP-(1–30).

Mechanism of GLP-1-induced increase of insulin binding in RINm5F cells. Competitive binding data revealed that GLP-1 increased 125I-insulin binding due to an increased receptor binding affinity and capacity. Figure 4 demonstrates a marked left shift of the competition-inhibition curves after 24 h incubation with GLP-1, indicating the improved binding affinity (IC50: 0.12 vs. 1.5 nM; Kd: 1.2 vs. 3.2 nM; GLP-1 vs. control, P < 0.05). As shown by Scatchard analysis (Fig. 5), the total number of insulin receptors concomitantly in-

**Fig. 1.** Time dependency of the effect of 1 nM glucagon-like peptide (GLP)-1 on specific 125I-labeled insulin binding in RINm5F cells. Specific 125I-insulin binding was determined from competitive binding studies by subtracting the amount of radioactivity bound nonspecifically in the presence of 1 μM unlabeled insulin, which was <3%. All binding data were corrected for nonspecific binding and tracer degradation. Data are means ± SE of 3 independent experiments. *P < 0.05 and **P < 0.005.

**Fig. 2.** Dose dependency of the GLP-1 effect on specific 125I-insulin binding in RINm5F cells. Cells were incubated for 2 h with 1 nM of GLP-1 alone or in combination with the GLP-1 receptor agonist exendin-4 or the GLP-1 receptor antagonist exendin-(9–39) amide (8, 17). GLP-1-(7–36) amide alone increased insulin binding from 100% (control) to 142 ± 6.3% (n = 17). Similar effects were observed with GLP-(7–37), which increased insulin binding to 141.7 ± 18.4% (n = 3). Incubation with exendin-4 (1 nM) alone stimulated insulin binding from 100% (control) to 161 ± 6% (P < 0.001; n = 6). The GLP-1 antagonist exendin-(9–39) amide alone showed no significant effects (116 ± 8%; n = 6). The GLP-1-induced increase in insulin binding (150 ± 10%) could be effectively blocked by 100 nM of exendin-(9–39) amide (116 ± 9%, P < 0.05 vs. GLP-1 alone; n = 6). As shown in Fig. 3, the dose-dependent increase in insulin binding observed with GLP-1-(7–36) amide was not seen with glucagon, GIP, or GIP-(1–30).

**Fig. 3.** Effects of GLP-1 and related peptides on 125I-insulin binding in RINm5F cells. Cells were incubated for 2 h with 1 and 100 nM of GLP-1, glucagon, GIP, and GIP-(1–30). Specific 125I-insulin binding was determined from competitive binding studies as described in MATERIALS AND METHODS. Mean values ± SE of 6–17 separate experiments are shown. ***P < 0.001.

**Fig. 4.** Competition-inhibition curves of 125I-insulin binding to RINm5F cells incubated with 1 nM GLP-1 or solvent for 24 h. Cells were incubated with 10 pM of 125I-insulin and various concentrations (10 pM-1 μM) of unlabeled insulin in assay buffer for 2 h at 15°C. GLP-1 induced a marked increase in insulin binding affinity. Data are means ± SE of 3 independent experiments. *P < 0.05, **P < 0.01, and ***P < 0.001.
GLP-1 improves insulin receptor binding

Effect of GLP-1 on insulin binding in RINm5F cells. Specific 125I-insulin binding was determined by subtracting the amount of radioactivity bound nonspecifically in the presence of 1 μM unlabeled insulin. Cells were preincubated with different concentrations of GLP-1 for different periods. Binding data were corrected for nonspecific binding and tracer degradation. Data are mean ± SE of 3 independent experiments. *P < 0.01 and **P < 0.001.

Discussion

GLP-1 regulates several functions in β-cells (6, 11). In the presence of elevated glucose levels, the peptide potently augments insulin secretion. Insulin production is increased by a direct effect on proinsulin gene transcription, and glucose-insensitive β-cells are rendered responsive by the peptide. GLP-1 regulates function and number of its own receptors on β-cells. Other hormones, e.g., somatostatin and leptin, have been shown to inhibit the effects of GLP-1. The present study demonstrates for the first time that GLP-1 improves insulin and proinsulin binding in insulinoma cells and in monocytes isolated from healthy and diabetic subjects. In RINm5F cells, GLP-1 exerts time- and dose-dependent effects, with 30% of the maximum already at concentrations of 20–50 pM, which are found in humans under physiological conditions (37). GLP-1 significantly increased both capacity and affinity of insulin binding. The stimulatory effect of GLP-1 on insulin binding occurred even within minutes. This suggests that GLP-1 may act on insulin receptors mainly located within or near the cell membrane rather than stimulating receptor de novo synthesis.

Finally, we assessed which intracellular pathway might be responsible for the GLP-1-induced increase in insulin binding. Therefore, we incubated RINm5F cells in HEPES assay buffer with the adenylyl cyclase activator forskolin (28), the stable cAMP analog cBiMPS (41), or the intracellular Ca2+ chelator BAPTA-AM (45). Control cells were incubated with the same volume of solvent alone. Two hours of incubation with forskolin (1 μM) or cBiMPS (100 nM) increased insulin binding capacity to 156 ± 15% or 145 ± 10% (P < 0.01 vs. controls; n = 6). In contrast, BAPTA-AM (10 μM) did not increase insulin binding (91 ± 5%; n = 6). Coincubation of BAPTA-AM with GLP-1 (1 nM) completely antagonized the stimulatory effect of GLP-1 on insulin receptor binding (92 ± 2%; P < 0.01 vs. GLP-1 alone; n = 6).

Effect of GLP-1 on insulin binding in monocytes. To address the question whether GLP-1 may affect insulin receptor binding in peripheral non-insulin-dependent tissues, we performed binding studies with mononuclear cells isolated from type 1 and type 2 diabetics and healthy control subjects. Scatchard analysis of binding data indicated two classes of binding sites (high-affinity/low-capacity and low-affinity/high-capacity), as in previous reports (5, 21, 24). As shown in Table 1, type 1 and type 2 diabetics showed a significantly lower number of high-capacity sites than control subjects (P < 0.05). Interestingly, GLP-1 (100 nM) yielded a significant increase in the number of high-capacity sites in both types of diabetes patients (P < 0.05). In isolated monocytes, we further compared the effects of 100 nM GLP-1 given for 24 h on the binding of insulin, proinsulin, and IGF-I. As shown in Table 2, proinsulin binding was significantly increased in all groups by GLP-1, whereas insulin binding increased in type 2 diabetics only. Interestingly, GLP-1 did not influence IGF-I binding.

DISCUSSION

GLP-1 regulates several functions in β-cells (6, 11). In the presence of elevated glucose levels, the peptide potently augments insulin secretion. Insulin production is increased by a direct effect on proinsulin gene transcription, and glucose-insensitive β-cells are rendered responsive by the peptide. GLP-1 regulates function and number of its own receptors on β-cells. Other hormones, e.g., somatostatin and leptin, have been shown to inhibit the effects of GLP-1. The present study demonstrates for the first time that GLP-1 improves insulin and proinsulin binding in insulinoma cells and in monocytes isolated from healthy and diabetic subjects. In RINm5F cells, GLP-1 exerts time- and dose-dependent effects, with 30% of the maximum already at concentrations of 20–50 pM, which are found in humans under physiological conditions (37). GLP-1 significantly increased both capacity and affinity of insulin binding. The stimulatory effect of GLP-1 on insulin binding occurred even within minutes. This suggests that GLP-1 may act on insulin receptors mainly located within or near the cell membrane rather than stimulating receptor de novo synthesis.
GLP-1 IMPROVES INSULIN RECEPTOR BINDING

Table 1. Effect of GLP-1 on number and affinity of insulin receptors on monocytes isolated from healthy subjects, type 1 diabetics, and type 2 diabetics

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Type 1 diabetics</th>
<th>Type 2 diabetics</th>
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<tbody>
<tr>
<td>No. of sites</td>
<td>953 ± 273</td>
<td>919 ± 281</td>
<td>840 ± 367</td>
</tr>
<tr>
<td>$K_D$, pM</td>
<td>81 ± 47</td>
<td>111 ± 41</td>
<td>74 ± 35</td>
</tr>
<tr>
<td>$K_D$, pM</td>
<td>1.259 ± 165</td>
<td>1.222 ± 386</td>
<td>1.764 ± 1,012</td>
</tr>
<tr>
<td>Specific binding, %/10^6 mononuclear cells</td>
<td>62 ± 4</td>
<td>124 ± 33</td>
<td>93 ± 68</td>
</tr>
<tr>
<td>$K_D$, nM</td>
<td></td>
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</table>

Data are means ± SE; $n = 4$ subjects in each group. GLP, glucagon-like peptide; *, without; †, with; $K_D$, dissociation constant. By Scatchard analysis, high-affinity/low-capacity binding sites could be distinguished from low-affinity/high-capacity sites. $^*P < 0.05$, diabetes vs. control. $^†P < 0.05$, −GLP-1 vs. +GLP-1.

From the changes in the shape of the Scatchard plots, it appeared that GLP-1 may lead to a conformational change of the insulin receptor that may be responsible for the improved binding affinity and capacity (5, 29, 47). GLP-1 increased the number of low-affinity/high-capacity sites, which in terms of their biological role are still a matter of debate (e.g., sparing receptors, insulin receptors mediating the mitogenic effects of insulin (5), binding sites for proinsulin/C peptide (20, 24)). GLP-1 also induced a significant increase in proinsulin binding. This is in accordance with our previous findings in IM-9 lymphoblasts (24) and the small intestinal crypt cell line IEC-6 (20), indicating that the low-affinity/high-capacity insulin binding sites are receptors for proinsulin. In isolated pancreatic islets of normal hamsters, proinsulin was shown to be more potent than insulin to decrease insulin secretion (7). In epidemiological studies, elevated proinsulin levels have been correlated with β-cell injury, obesity, type 2 diabetes, and cardiovascular mortality (13, 25). Although the role of proinsulin in atherogenesis is still a matter of debate [e.g., sparing receptors, Ca2+ influx, Ca2+ mobilization, and phosphorylation of downstream signaling proteins could show different kinetics when induced by GLP-1, GIP, and glucagon; third, the cross talk between G protein-coupled pathways and tyrosine kinases (e.g., insulin receptor) may be different for GLP-1, GIP, or glucagon (15, 46)].

Table 2. Effects of GLP-1 on binding of insulin, proinsulin, or IGF-I on monocytes isolated from healthy subjects, type 1 diabetics, and type 2 diabetics

<table>
<thead>
<tr>
<th></th>
<th>−GLP-1</th>
<th>+GLP-1</th>
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<tbody>
<tr>
<td>Insulin binding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.83 ± 0.15</td>
<td>0.94 ± 0.12</td>
</tr>
<tr>
<td>Type 1 diabetics</td>
<td>0.75 ± 0.17</td>
<td>0.91 ± 0.19</td>
</tr>
<tr>
<td>Type 2 diabetics</td>
<td>0.57 ± 0.14</td>
<td>0.93 ± 0.21</td>
</tr>
<tr>
<td>Proinsulin binding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.04 ± 0.02</td>
<td>0.17 ± 0.05†</td>
</tr>
<tr>
<td>Type 1 diabetics</td>
<td>0.21 ± 0.16</td>
<td>0.52 ± 0.21</td>
</tr>
<tr>
<td>Type 2 diabetics</td>
<td>0.06 ± 0.04</td>
<td>0.32 ± 0.07†</td>
</tr>
<tr>
<td>IGF-I binding</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.35 ± 0.08</td>
<td>0.33 ± 0.07</td>
</tr>
<tr>
<td>Type 1 diabetics</td>
<td>0.25 ± 0.06</td>
<td>0.36 ± 0.08</td>
</tr>
<tr>
<td>Type 2 diabetics</td>
<td>0.31 ± 0.04</td>
<td>0.42 ± 0.05</td>
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Data are means ± SE; $n = 4$ subjects in each group. IGF-I, insulin-like growth factor I. Cells were incubated for 2 h at 15°C with 10 pM of [125I]-labeled insulin, proinsulin, or IGF-I with and without 1 μM of unlabeled homologues peptides. Specific [125I]-peptide binding (%) was determined by subtracting the amount of radioactivity bound nonspecifically in the presence of 1 μM unlabeled homologous peptide. $^*P < 0.05$, diabetes vs. control. $^†P < 0.05$, −GLP-1 vs. +GLP-1 diabetes.
Our observation that GLP-1 but not GIP increases insulin binding may be of clinical relevance and may explain why GLP-1 preserved its activity as an incretin hormone in patients with type 2 diabetes mellitus, whereas GIP did not (33). GLP-1 not only acts as an incretin hormone stimulating postprandial insulin secretion but also enhances peripheral glucose metabolism. The mechanisms underlying the latter effect of GLP-1 remain unclear. It has been suggested that GLP-1 may improve insulin sensitivity in healthy subjects and in diabetic patients (4, 18). Hyperinsulinemic, euglycemic clamp studies in healthy humans showed that GLP-1-(7–36) amide administered for 3 h, leading to circulating levels within the physiological range, does not affect insulin sensitivity (39). It is possible that the conventional techniques for assessing insulin sensitivity in vivo are not sensitive to detect the effect of GLP-1 on insulin binding. Although GLP-1 receptors are reported in a wide variety of tissues (6, 11), it could be possible that the effect of GLP-1 is limited to pancreatic β-cells and not to tissues that comprise the bulk of measured whole body insulin sensitivity. As recently discussed, some controversy remains with regard to the expression of GLP-1 receptors in peripheral tissues (6). Structural variants of the GLP-1 receptor or a second closely related receptor may be expressed in different tissues. Our binding data in monocytes do not completely exclude the existence of GLP-1 receptors. It should be noted that peptide degradation was underestimated by the TCA method, which is suitable to demonstrate gross degradation of peptides but will not give any indication of degradation limited to only a few amino acids. Fragmented GLP-1 may have disturbed the ability of unlabeled GLP-1 to displace the radioligand. GLP-1 is especially susceptible to degradation by dipeptidyl peptidase IV (present on monocytes), which removes a dipeptide from the NH₂-terminus of the molecule. The resultant metabolite can still bind to the GLP-1 receptor but is unable to transduce a signal because of the loss of the NH₂-terminal dipeptide. This may explain why in monocytes higher concentrations of GLP-1 were needed than in RINm5F cells to modulate insulin and proinsulin binding.

The binding studies in isolated monocytes, a model for the human insulin receptor on peripheral tissues (1, 21, 22), are consistent with the hypothesis that GLP-1 modulates insulin receptor binding. We demonstrated that incubation of isolated monocytes with GLP-1 differentially affected the two types of insulin binding sites. According to our previous findings (21, 22), the low-affinity/high-capacity binding sites were diminished in type 1 and type 2 diabetes but were significantly increased after incubation with GLP-1. It has been elucidated that this binding type is mainly involved in signaling the metabolic activity of insulin (5). The type 2 diabetes were poorly matched in terms of age and body mass index. However, when we investigated insulin binding in a larger cohort of subjects (unpublished observations), we found no significant influence of age, whereas a high body mass index was associated with lower insulin binding. Our data further demonstrate that, in monocytes of control subjects and diabetics, GLP-1 significantly increases proinsulin binding but not IGF-I binding. This underlines the specificity of GLP-1 action and indicates that the high-capacity/low-affinity insulin binding sites are not functional IGF-I receptors.

In summary, our findings in insulinoma cells and in circulating mononuclear cells demonstrate that GLP-1 specifically increases the number of high-capacity/low-affinity insulin binding sites that may be functional proinsulin receptors. Further studies are necessary to elucidate whether the feedback regulation of insulin and proinsulin secretion and the increase of insulin-independent glucose disposal could be attributed to the GLP-1 effect on receptor binding of insulin and proinsulin.

We thank E. Rüber for excellent technical assistance. This work was supported by the Landesforschungsschwerpunkt Baden-Württemberg: Spätfolgen bei Diabetes Mellitus (to R. D. Fuusgsenger) and the Landesforschungsschwerpunkt Baden-Württemberg: Modulation von Wachstumsfaktoren als Therapieprinzip (to P. M. Jehle).

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