Mouse Pancreatic β-Cells Exhibit Preserved Glucose Competence After Disruption of the *Glucagon-Like Peptide-1 Receptor* Gene

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Previous work suggested that glucagon-like peptide 1 (GLP-1) can acutely regulate insulin secretion in two ways, *1*) by acting as an incretin, causing amplification of glucose-induced insulin release when glucose is given orally as opposed to intravenous glucose injection; and *2*) by keeping the β-cell population in a glucose-competent state. The observation that mice with homozygous disruption of the *GLP-1 receptor* gene are diabetic with a diminished incretin response to glucose underlines the first function in vivo. Isolated islets of Langerhans from *GLP-1 receptor* –/– mice were studied to assess the second function in vitro. Absence of pancreatic GLP-1 receptor function was observed in *GLP-1 receptor* –/– mice, as exemplified by loss of [^{125}I]GLP-1 binding to pancreatic islets in situ and by the lack of GLP-1 potentiation of glucose-induced insulin secretion from perifused islets. Acute glucose competence of the β-cells, assessed by perifusing islets with stepwise increases of the medium glucose concentration, was well preserved in *GLP-1 receptor* –/– islets in terms of insulin secretion. Furthermore, neither islet nor total pancreatic insulin content was significantly changed in the *GLP-1 receptor* –/– mice when compared with age- and sex-matched controls. In conclusion, mouse islets exhibit preserved insulin storage capacity and glucose-dependent insulin secretion despite the loss of functional GLP-1 receptors. The results demonstrate that the glucose responsiveness of islet β-cells is well preserved in the absence of GLP-1 receptor signaling. *Diabetes* 47:646–652, 1998

Pancreatic β-cells require co-stimulation with nutrients and (neuro)hormones for the physiological control of insulin release (1–4). Part of the hormonal control of insulin release proceeds via receptor-mediated regulation of cAMP production in β-cells (5,6). Cyclic AMP synergizes with signals derived from glucose metabolism at various possible levels, including enhancement of voltage-dependent influx of calcium (7,8) and calcium-independent sensitization of exocytosis (9). The physiological relevance of this synergism between glucose and cAMP is underlined by the gluco-incretins glucagon-like peptide-1(7-36) amide (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which are responsible for a greater insulin secretory response after oral glucose than after intravenous glucose (10–16). Several studies have underlined the importance of GLP-1 as an incretin. First, GLP-1 was found to be a potent stimulator of insulin secretion, both in rodents (11) and in humans (12); the peptide bound to a specific class of high-affinity receptors (13) that were present in rat β-cells (6). Second, occupation of the GLP-1 receptor (GLP-1R) with the antagonist exendin(9-39) amide (14) blocked at least part of the incretin effect in rats (15). Third, mice with homozygous disruption of the *GLP-1 receptor* gene (*GLP-1R* –/– mice; 16) exhibited disturbed glucose tolerance with high plasma glucose and low plasma insulin levels, emphasizing the importance of GLP-1R in glucose homeostasis. However, the in vivo studies using exendin-(9-39)-amide or *GLP-1R* –/– mice are complex, in that loss of β-cell stimulation is one of several mechanisms by which glucose homeostasis can be disturbed, considering that GLP-1 receptors have been implicated in the hypothalamic control of food intake (17), in the control of gastric emptying (18), and in peripheral glucose uptake (19). Furthermore, in addition to its effect as an incretin, it was proposed that GLP-1 can regulate β-cell function by maintaining at least part of the β-cell population in a glucose-competent state, allowing the cells to respond to glucose in terms of hormone secretion (20). This concept merits further investigation, because individual pancreatic β-cells have been reported to be heterogenous in their responsiveness to glucose, in terms of insulin biosynthesis (21), insulin release (22), and cytoplasmic calcium (23). The present in vitro study on islets of Langerhans isolated from diabetic GLP-1R–deficient mice was undertaken with the aim to assess the functional consequence of *GLP-1R* disruption in the endocrine pancreas. The results show that glucose-induced insulin secretion in pancreatic islets obtained from GLP-1R–deficient mice is well preserved at the age of 8–10 weeks, demonstrating that the GLP-1R per se is not required for maintaining glucose competence in pancreatic β-cells.

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BSA, bovine serum albumin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1(7–36) amide; GLP-1R, GLP-1 receptor; RT-PCR, reverse transcription–polymerase chain reaction; SSC, sodium chloride–sodium citrate buffer.

RESEARCH DESIGN AND METHODS

Animals. The study was conducted on 8- to 10-week-old male and female CD-1 mice fed ad libitum that were wild type (+/+) or homozygous (–/–) for the targeted

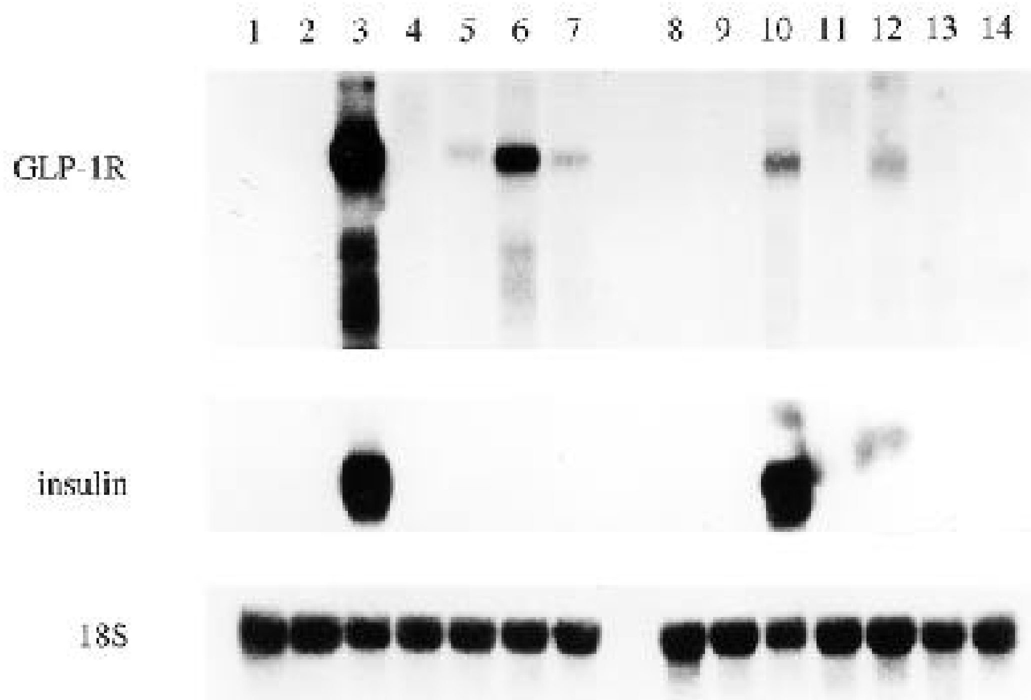

FIG. 1. GLP-1R and insulin mRNA in islets from *GLP-1R* +/+ and –/– mice. Total RNA (40 µg) from the following tissues from *GLP-1R* +/+ mice (*lanes 1–7*) and *GLP-1R* –/– mice (*lanes 8–14*) was loaded: liver (*lanes 1*, *8*), kidney (*lanes 2*, *9*), pancreatic islets (*lanes 3*, *10*), heart (*lanes 4*, *11*), brain (*lanes 5*, *12*), lung (*lanes 6*, *13*), and stomach (*lanes 7*, *14*). Exposure times were 2 days (GLP-1R), 4 h (insulin), and 1 h (18S rRNA).

null mutation in the *GLP-1R* gene (16). The animals were bred under specific pathogen–free conditions and cared for according to Belgian regulations of animal welfare. Body weight at age 8 weeks was not influenced by the –/– mutation: data for male *GLP-1R +/+* mice (34.1 ± 0.8 g) versus –/– mice (33.3 ± 0.7 g) and for female *GLP-1R +/+* mice (24.4 ± 0.6 g) versus –/– mice (25.0 ± 0.9 g) were comparable (mean ± SE of six animals per sex per strain). Plasma glucose levels, determined under nonfasting conditions in 13 mice per sex per strain, were as follows: female *GLP-1R +/+* mice (6.7 ± 0.3 mmol/l) versus *GLP-1R –/–* mice (6.3 ± 0.2 mmol/l); male *GLP-1R +/+* mice (7.1 ± 0.5 mmol/l) versus *GLP-1R –/–* mice (7.4 ± 0.4 mmol/l). Thus, no difference of blood glucose and body weight was observed in the age window of 8–10 weeks.

Isolation and culture of mouse pancreatic islets. Islets of Langerhans were isolated using collagenase P (Boehringer Mannheim, Mannheim, Germany). The pancreases of the decapitated animals were injected in situ with cold islet isolation medium (0.10 mol/l NaCl, 4.7 mmol/l KCl, 1.8 mmol/l $CaCl_2$, 20 mmol/l $NaHCO_3$, 1.2 mmol/l KH_2PO_4, 1.2 mmol/l $MgSO_4$, 16 mmol/l HEPES, 2.8 mmol/l glucose, and 1% bovine serum albumin [BSA], pH 7.4), followed by removal of the glands. Pancreases were digested for 30 min at 37°C in a shaking incubator using islet isolation medium containing 2 mg/ml of collagenase, yielding approximately 200 islets per pancreas. After isolation, islets were cultured overnight in F10 medium Nutrient Mixture (Ham) (GIBCO BRL, Life Technologies, Strathclyde, U.K.) containing 0.075 mg/ml penicillin, 0.1 mg/ml streptomycin, 0.5% (wt/vol) BSA (fraction V, RIA grade; Sigma, St. Louis, MO), 2 mmol/l glutamine, and 10 mmol/l glucose (24).

Analysis of GLP-1 receptor mRNA. Total RNA was isolated from different tissues (liver, kidney, stomach, heart, brain, lung), and 3,000 pooled pancreatic islets were obtained from decapitated ad-libitum-fed *GLP-1R +/+* and –/– mice by the TRIzol method (GIBCO BRL Life Technologies, Paisley, U.K.). The islets were separated according to size on 1.5% agarose gels (40 µg per lane). Blots were ultraviolet cross-linked and hybridized with a ^{32}P-labeled mouse 1.5 Kbp GLP-1 receptor cDNA probe that was isolated from a mouse lung library (Stratagene, La Jolla, CA). The final stringent washing step was performed for 30 min at 60°C in 2.5% sodium dodecyl sulfate and 0.5× sodium chloride–sodium citrate (SSC). Autoradiographic exposure was done for 2 days to a Biomax MS film (Amersham, Aylesbury, U.K.). Stripped blots were rehybridized with a rat ins-1 cDNA probe (25) to assess islet insulin mRNA abundance. After a final stringent wash in 0.1× SSC and 2.5% sodium dodecyl sulfate at 60°C, the films were exposed to Biomax MS film and developed after 4 h. Integrity and load of total RNA was assessed by rehybridizing the stripped blots to a oligonucleotide probe for rat 18 S RNA with the following sequence: 5′-CTT CTT CTA GAT AGT CAA GTT CGA CCG TCT-3′ (nucleotides 1802–1832). After a final stringent washing step in 0.2× SSC and 2.5% sodium dodecyl sulfate at 54°C, the blot was exposed for 1 h to a Biomax MS film. The intensity of the signals on autoradiographic films were scanned on an Ultroscan XL densitometer (Pharmacia, Uppsala, Sweden). Values used for calculations were deduced from the integrated area under the curve.

For the analysis via reverse transcription–polymerase chain reaction (RT-PCR), total RNA (0.4 µg) from liver, brain, lung, and islets of Langerhans of *GLP-1R –/–* and *+/+* mice was reverse transcribed and amplified according to an adapted protocol of the GeneAmp RNA-PCR kit (Perkin Elmer/Cetus, Emeryville, CA) using Taq Gold polymerase (Perkin Elmer). Specific primers were designed to anneal with codon 160–167/388–394 of mouse GLP-1R cDNA and codon 249–255/338–344 for β-actin cDNA, yielding expected PCR products of 705 and 288 bp. respectively. The cycling profile for GLP-1R was 9 min at 94°C, followed by 0.5 min at 94°C, 1 min at 59°C, and 1 min at 72°C for the first 5 cycles; 0.5 min at 94°C, 1 min at 56°C, and 1 min at 72°C for 30 cycles; and a 10-min extension at 72°C before cooling to 4°C. The cycling profile of β-actin was the same except the annealing temperature, which was 62°C for the first 5 cycles and 60°C for the other 30 cycles. PCR products were controlled for their length on 2% agarose and sequenced by fluorescent cycle sequencing on an ABI Prism 310 Genetic Analyzer (Perkin Elmer). The complete sequence of mouse GLP-1R cDNA was deposited in the EMBL nucleotide sequence database (accession number AJ001692).

In situ binding of [^{125}I]GLP-1. The preparation of [^{125}I]GLP-1 was based on a chloramine-T method. Ten micrograms of GLP-1 (Sigma) was first mixed with 25 µl phosphate buffer (0.1 mol/l, pH 7.6) and 10 µl Na^{125}I (100 mCi/ml, IMS-30, Amersham, Buckinghamshire, U.K.) before exposure to 25 µl chloramine-T (4.2 µg/25 µl in 0.02 mol/l phosphate buffer, pH 7.6). The oxidation reaction proceeded at 4°C under continuous stirring and was stopped after 90 s by the addition of 25 µl $Na_2S_2O_5$ (400 µg/ml in 0.02 mol/l phosphate buffer, pH 7.6). During the reaction, more than 85% of Na^{125}I was incorporated into the peptide. The radiolabeled ligand had a specific activity of approximately 17 Bq/fmol. Pancreases from male *GLP-1R +/+* and –/– mice were snap-frozen in a mixture of isopentane and carbonic acid ice. The tissue was sectioned in a cryostat in sets of 5 subsequent slices of 6 µm and collected on coated slides. One section was stained with hematoxylin and two sections were analyzed for the presence of immunoreactive glucagon and immunoreactive insulin, respectively. The two remaining sections were preincubated in Ca^{2+}-free Krebs-Ringer buffer (pH 7.4) containing 10 mmol/l HEPES, 1 mmol/l EGTA, 2.8 mmol/l glucose, 1% BSA, 0.8 mg/ml Bacitracin (Sigma), and Trasylol (10^4 kallikrein inhibiting units/ml; Bayer, Leverkusen, Germany) for 20 min to remove endogenous ligand. Excess liquid was drained from the slides, which were then incubated for 90 min at room temperature with 200

μl Krebs-Ringer buffer containing [^{125}I]GLP-1 (1.5×10^5 cpm/ml). Nonspecific binding was determined in the presence of 0.2 μmol/l exendin(9-39) amide. The slides were washed 4 times for 30 s in ice-cold Krebs-Ringer buffer and 2 times in ice-cold water. The ligand was fixed to the receptor by briefly incubating in 2% glutaraldehyde. After being dipped in 80% ethanol, the slides were dried and dipped into K2 emulsion (Ilford Limited Mobberley, Cheshire, U.K.). After 3 weeks' exposure at 4°C, the slides were developed and briefly counterstained with hematoxylin.
Insulin release from isolated islets. The effects of glucose and GLP-1 on insulin release were assessed in an islet perifusion system (22) using a multiple microchamber module (Endotronics, Coon Rapids, MN) with pump and thermostat, with islets from one *GLP-1R* –/– pancreas being perifused in parallel with islets obtained from one *GLP-1R* +/+ control animal. Samples were collected every minute and assayed for immunoreactive insulin in a radioimmunoassay (2), with the dead space of the perifusion system being taken into account when expressing results in function of time. Preperifusion was done for 20 min on Biogel P2 columns (Biorad) using F10 medium Nutrient Mixture (Ham) supplemented with 0.5% BSA, 2 mmol/l glutamine, 2 mmol/l $CaCl_2$, and 1.4 mmol/l glucose medium equilibrated with 95% O_2/5% CO_2, with the flow rates at 0.5 ml/min. The first experiment (female mice) consisted of exposure to 1.4 mmol/l glucose (20 min), 20 mmol/l glucose (30 min), 20 mmol/l glucose with 10^{-8} mol/l GLP-1 (30 min), 20 mmol/l glucose with 10^{-8} mol/l GLP-1 and 10^{-6} mol/l exendin(9-39) amide (20 min), and, again, 1.4 mmol/l glucose (20 min). In the second experiment, male islets of *GLP-1R* +/+ and –/– mice were exposed in parallel to a sequence of 15-min pulses of increasing glucose concentrations, which alternated with 15-min periods of 2.8 mmol/l glucose, as described in detail previously (22). At the end of each perifusion experiment, the islets in the perifusion columns were extracted in 2 mol/l acetic acid and 0.25% BSA and assayed for immunoreactive insulin content.
Measurement of total pancreatic and islet insulin content. Pancreases were dissected from ad-libitum-fed female and male *GLP-1R* +/+ or –/– mice (10 weeks old) and stored in liquid nitrogen until use. The pancreases were homogenized via ultrasound in 15 ml of 2 mol/l acetic acid containing 0.25% BSA, incubated for 2 h on ice, and centrifuged at $8{,}000g \times$ min. The supernatant fraction was collected while the pellet was sonicated again and centrifuged at $8{,}000g \times$ min. The second supernatant fraction was pooled with the first for analysis of insulin content as determined by an insulin radioimmunoassay (2). Islet insulin content was measured after each perifusion experiment by sonicating the Biogel P2 containing approximately 200 islets in 5 ml of 2 mol/l acetic acid containing 0.25% BSA.
Statistical analysis. Significance of differences between conditions was tested by Student's t tests. Data are expressed as mean values ± SE of n independent experiments.

RESULTS

Characterization of GLP-1 receptor mRNA in *GLP-1R* –/– and *GLP-1R* +/+ mice. Northern blot analysis of different tissues obtained from *GLP-1R* +/+ mice showed that GLP-1R expression was high in lung and pancreatic islets and lower, but clearly detectable, in the brain and stomach (Fig. 1). A low signal level was found in the kidney and heart, whereas no mRNA was detected in the liver, even after prolonged exposure. GLP-1 receptor transcripts of similar length could be detected in brain and pancreatic islets from *GLP-1R* –/– mice, but the abundance was much lower than in the control mice. In contrast, the estimated amount of insulin mRNA appeared the same in *GLP-1R* –/– and *GLP-1R* +/+ mice (Fig. 1). Densitometric analysis of blots from three independent experiments confirmed this impression, as the following signal intensity ratios (*GLP-1R* +/+ mice over *GLP-1R* –/– mice) were obtained: 6 ± 1.5 (islet GLP-1R mRNA, significantly different from 1, $P < 0.05$), 1.1 ± 0.3 (islet preproinsulin mRNA), and 1.1 ± 0.07 (islet 18 S rRNA). Therefore, the loss of GLP-1 action in *GLP-1R* –/– mice is not paralleled by a general reduction of β-cell gene expression or reduced islet RNA.

More detailed characterization of the GLP-1R transcripts extracted from the liver, lung, brain, and pancreatic islets in *GLP-1R*+/+ and –/– mice was performed via RT-PCR. Primers were designed to span the 315-bp region that was predicted to be deleted by the homologous recombination at the GLP-1R gene (Fig. 2*A*). As shown in Fig. 2*B*, a smaller PCR product of the predicted size (390 bp) was obtained after amplification of lung, pancreatic islet, and brain cDNA from *GLP-1R* –/– mice, whereas the PCR product from *GLP-1R* +/+ mice migrated as a larger band (705 bp). The nucleotide sequence of the 390 bp cDNA fragment that was amplified from *GLP-1R* –/– islets was 100% identical to the corresponding fragment of the full-length GLP-1R cDNA cloned from a mouse lung cDNA

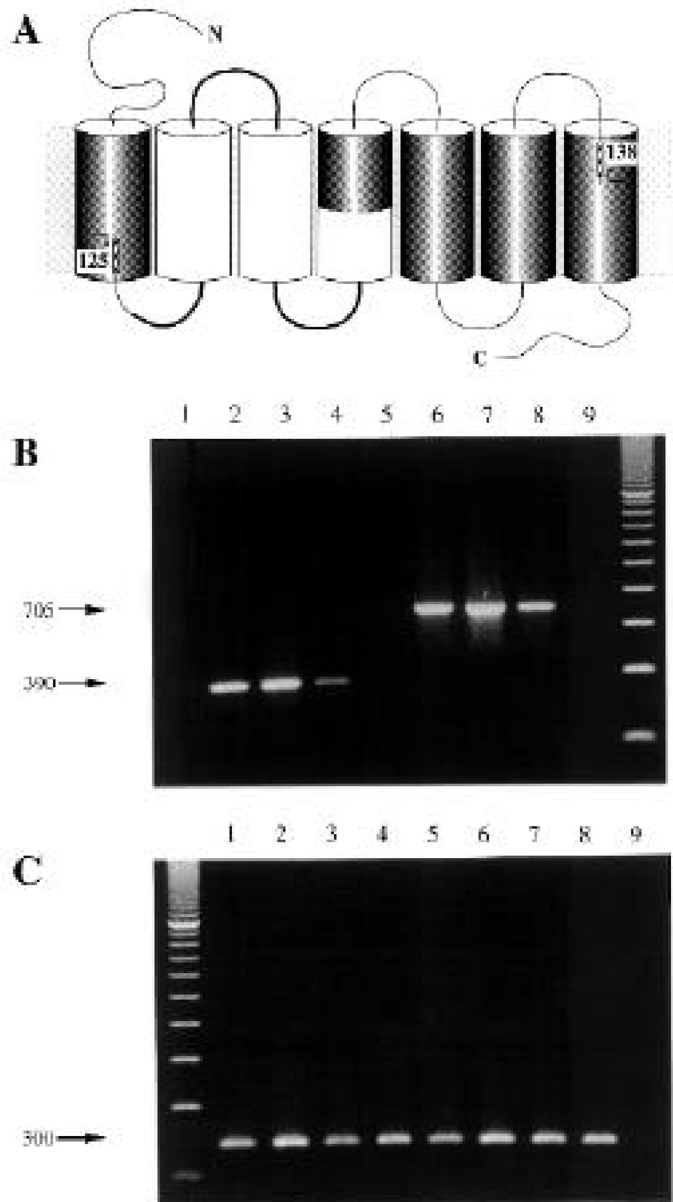

FIG. 2. RT-PCR of GLP-1 receptor transcripts in *GLP-1R* +/+ and –/– mice. *A*: Model of the receptor protein, indicating the 7-helix structure and the truncation after recombination of the *GLP-1R* gene (bold lines and the white transmembrane helices 2, 3, and 4). The regions corresponding to the location of the primer pair (125–138) for RT-PCR are indicated by the hatched boxes. *B*: Amplification of GLP-1R cDNA using primer set 125–138. *Lanes 1–4*, *GLP-1R* –/– mice; *lanes 5–8*, *GLP-1R* +/+ mice. cDNA was obtained from the following tissues: liver (*lanes 1*, *5*), lung (*lanes 2*, *6*), pancreatic islets (*lanes 3*, *7*), and brain (*lanes 4*, *8*). *Lane 9* represents amplification using water instead of RNA. Right lane: 200 bp molecular weight standards. *C*: Amplification of β-actin cDNA from the same tissues as in *B*. Left lane: 200 bp molecular weight standards.

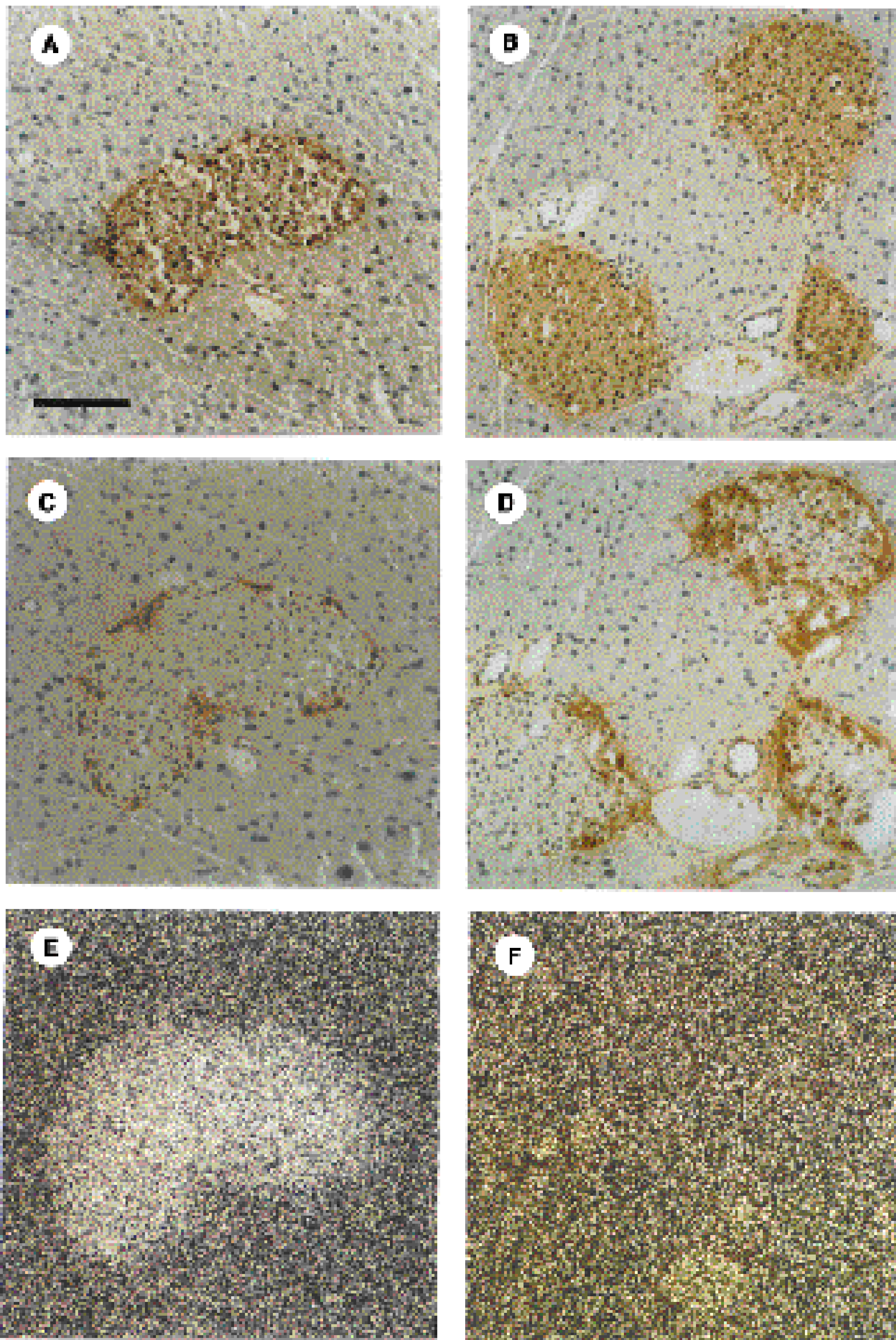

FIG. 3. In situ binding of [^{125}I]GLP-1 to mouse pancreatic sections. Consecutive 6-µm pancreatic sections from male *GLP-1R* +/+ (*A,C,E*) and *GLP-1R* –/– (*B,D,F*) mice were immunostained with insulin (*A,B*) and glucagon (*C,D*) to localize islet β-cells and α-cells respectively, and exposed to [^{125}I]GLP-1 for in situ binding (dark field epipolarization; *E,F*). Binding specificity was tested by incubating the sections simultaneously with [^{125}I]GLP-1 and 200 nmol/l exendin(9-39) amide, which resulted in background signals over the islets of *GLP-1R* +/+ mice (data not shown). The scale bar in *A* represents 100 µm.

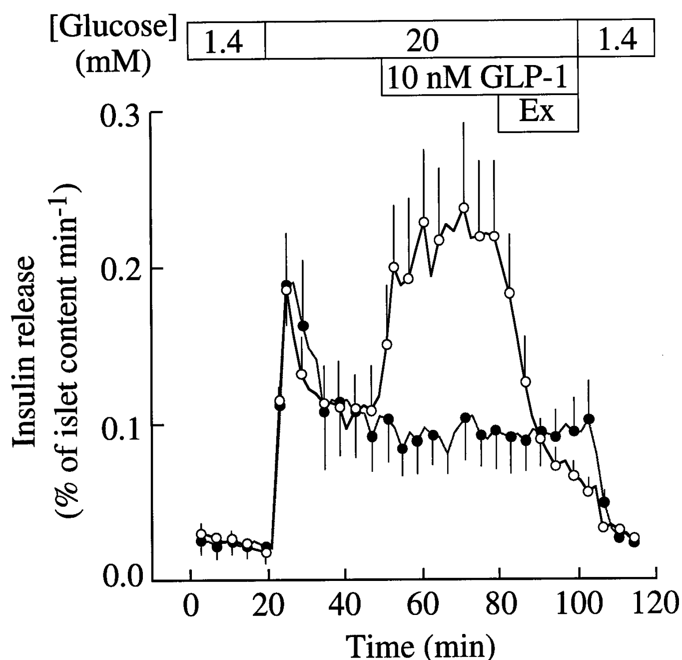

FIG. 4. Effect of GLP-1 on 20 mmol/l glucose-induced insulin release. Islets from female mice were perifused with glucose alone, 20 mmol/l glucose plus 10 nmol/l GLP-1, and 20 mmol/l glucose plus 10 nmol/l GLP-1 plus 1 µmol/l exendin(9-39) amide. Data represent mean values ± SE of four experiments from *GLP-1R +/+* mice (○) and *GLP-1R –/–* mice (●). For graphical clarity, mean symbols and error bars are shown once per four consecutive minute fractions.

library (data not shown) and confirmed the presence of a deletion of 315 bp that comprise nucleotides encoding the second to fourth transmembrane helices and the intervening intra- and extracellular loops (Fig. 2*A*).

Analysis of in situ GLP-1 binding in *GLP-1R –/–* and *GLP-1R +/+* mice. As the smaller GLP-1R transcript might encode a truncated receptor still capable of binding GLP-1 (Fig. 2*A*), we compared both binding properties and functional activity of the truncated and wild-type GLP-1 receptors (Figs. 3 and 4). In situ binding was performed with pancreatic sections from *GLP-1R +/+* and *–/–* mice. Consecutive sections were immunostained for insulin (Fig. 3*A* and *B*) and glucagon (Fig. 3*C* and *D*) to identify islet β-cells and α-cells, respectively. As shown in Fig. 3*E*, [^{125}I]GLP-1 binding was homogeneously distributed over the islet area of *GLP-1R +/+* mice. Specificity of [^{125}I]GLP-1 binding was assessed by incubating the sections simultaneously with an excess of exendin(9-39) amide, which reduced binding to background levels (data not shown). In *GLP-1R –/–* islets, no [^{125}I]GLP-1 binding was detected above the background signal (Fig. 3*F*).

Insulin release from perifused islets. To assess the functional consequences of the expression of truncated GLP-1 receptors in islets, the effect of 10 nmol/l GLP-1 on insulin secretion was studied at 20 mmol/l glucose (Fig. 4). In the *GLP-1R +/+* islets, the rate of insulin release at 20 mmol/l glucose (0.11 ± 0.03% of islet content · min^{-1}) increased twofold after adding 10 nmol/l GLP-1 (0.22 ± 0.05% of content · min^{-1}; means ± SE; n = 4; P < 0.02). In contrast, the potentiating effect of GLP-1 was absent in the *GLP-1R –/–* mice. The reversibility and specificity of the stimulatory effect of GLP-1 was shown by adding 1 µmol/l exendin(9-39) amide during the last 20 min of GLP-1 stimulation. In the *GLP-1R +/+* mice, the antagonist completely blocked the effect of GLP-1, whereas in the *GLP-1R –/–* islets, exendin(9-39) amide had no effect on insulin secretion. Remarkably, the data in Fig. 4 indicate that glucose-induced insulin release was well preserved in the *GLP-1R –/–* islets, despite the loss of functional GLP-1 receptors. To further assess the glucose responsiveness of *GLP-1R –/–* β-cells, isolated islets from knockout and control mice were stimulated for 15 min with different glucose concentrations in a perifusion system (22). The secretory response to high (22 mmol/l) glucose was identical in the *GLP-1R +/+* and *–/–* islets (Fig. 5). Furthermore, the responsiveness to intermediate glucose concentrations was comparable in the islets from *GLP-1R –/–* and *GLP-1R +/+* mice, both in terms of dose-dependence and in terms of the total amount of insulin that was secreted (Table 1).

Measurement of pancreatic insulin content. Differences in islet insulin content compensating for differences in insulin release were not responsible for the similarity in the secretory response of *GLP-1R –/–* and *+/+* islets (Table 2). To exclude the influence of a selection bias during the islet handpicking after collagenase digestion of the pancreas, we measured insulin content of intact pancreases that were dissected from ad-libitum-fed animals (Table 2). Overall insulin content, expressed per gram wet weight, was the same in pancreases from 10-week-old *GLP-1R –/–* mice as in those from *GLP-1R +/+* mice. Furthermore, the insulin content was the same in male and female animals in all groups (Table 2).

DISCUSSION

In patients with NIDDM, infusion of GLP-1 ameliorates glucose homeostasis (26), an effect that is not achieved to the same extent with GIP (27). In the GLP-1–treated patients, reduced plasma glucose was observed in parallel to an increased insulin:glucose ratio and reduced plasma glucagon levels (26); hence it can be considered that the therapeutic effect of GLP-1 involves a direct interaction with the endocrine pancreas. The present study has investigated the latter interaction in an animal model of GLP-1R deficiency (16), addressing the question whether loss of GLP-1R function in pancreatic β-cells influences the responsiveness of the cells to glucose. For this purpose, we have isolated mouse islets of Langerhans and studied their insulin secretory capacity in a dynamic perifusion system. The recombination event within the *GLP-1R* gene leads to the expression of a truncated GLP-1R mRNA and to a complete loss of GLP-1R activity.

The islets used in our studies were isolated from 8- to 10-week-old animals; at that age, the mice exhibit abnormal glucose tolerance, with increased plasma glucose:insulin ratios and defective insulin secretion when challenged with oral or peritoneal glucose (16). This relatively early age was chosen for the present study to avoid late secondary islet dysfunction that might occur as a consequence of chronic hyperglycemia. Northern blot analysis of total RNA extracted from various tissues of *GLP-1R –/–* mice showed the presence of read-through GLP-1 transcripts in brain, lung, and pancreatic islets, but the measured abundance in the islets was sixfold lower than in islets of *GLP-1R +/+* control mice. The relative difference in levels of mRNA transcripts may reflect a difference in gene transcription, or more likely, the truncated GLP-1R –/– transcript may be less stable than the wild-type transcript, as has been observed for other gene products

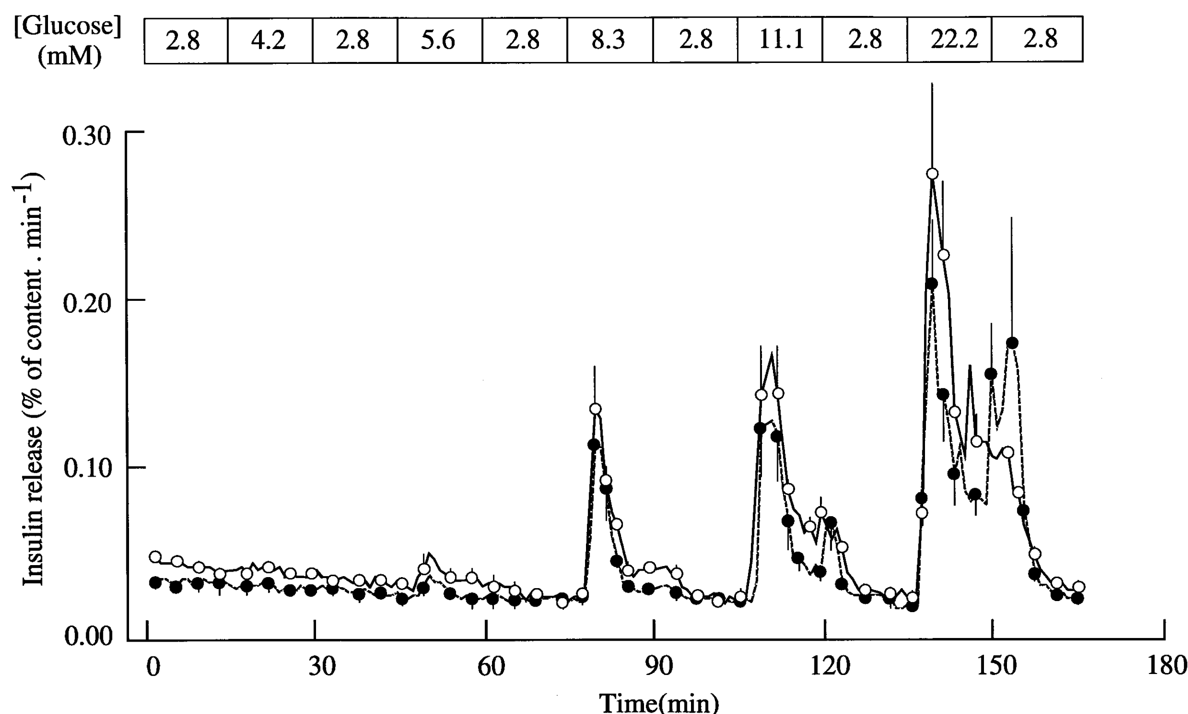

FIG. 5. Dose-dependent glucose-induced insulin release from *GLP-1R* +/+ and –/– islets. Islets from male mice were perifused for 15-min periods with elevated glucose at the indicated concentrations, alternating with basal medium (2.8 mmol/l glucose). Data represent mean values ± SE of five experiments in *GLP-1R* +/+ mice (○) and in *GLP-1R* –/– mice (●). For graphical clarity, mean symbols and error bars are shown once per four consecutive minute fractions.

(28). The 315-bp gene deletion results in a transcript with a preserved open reading frame. It is presently unknown whether the truncated receptor mRNA is effectively translated into protein and, if so, whether the protein is appropriately targeted to the plasma membrane. Even if the truncated receptor protein is correctly targeted to the plasma membrane, it might be predicted that GLP-1 binding would be abnormal (Fig. 2*A*), in that a large part of the putative ligand-binding domain is absent from the truncated protein. In accordance with this idea, in situ receptor binding of [^{125}I]GLP-1 on pancreatic sections was negative, whereas in pancreatic sections of control animals, [^{125}I]GLP-1 bound specifically to the islets of Langerhans. Taken together with the total lack of insulin secretion following GLP-1 stimulation (Fig. 4), the available evidence strongly supports the fact that the homozygous recombinant mice do not express functional GLP-1 receptors in their islets of Langerhans.

Despite the importance of GLP-1 for the control of β-cell function (6,11–13,20), it is remarkable that glucose-induced insulin release was quite well preserved in *GLP-1R* –/– islets compared with control mice (Fig. 5, Table 1). Thus, although addition of GLP-1 to isolated β-cells induced glucose competence in vitro (20), signal transduction through the GLP-1 receptor seems not absolutely required for this induction, at least in mice. It is possible that part of the diabetic phenotype in *GLP-1R* –/– mice (16) is due to defects in previously described extrapancreatic actions of GLP-1 (17–19). However, a major hypothalamic contribution to the overall phenotype is unlikely, because the knockout and control animals exhibit the same body weights (this study and 16). Alternatively, compensation for the loss of islet GLP-1R in the *GLP-1R* –/– mice may be an explanation for the preserved response to glucose. Because mammalian pancreatic islets produce glucagon and express both glucagon receptors and GIP receptors that—in addition to the GLP-1 receptors—stimulate cAMP production and insulin release (6,29), it is possible that increased glucagon and/or GIP signaling partly compensates for the loss of GLP-1 receptors. Further study of the mechanisms underlying glucose-induced insulin secretion using GLP-1R –/– islets may provide additional insights into the factors necessary for induction and/or maintenance of glucose competence, both in vitro and in vivo.

In summary, the data presented in this paper demonstrate that disruption of GLP-1 signaling is not associated with a major perturbation in glucose-induced insulin secretion from isolated islets of Langerhans. It remains to be assessed via which mechanisms this can be achieved.

TABLE 1
Glucose-induced insulin release from *GLP-1R* +/+ and –/– islets

Glucose during perifusion (mmol/l)	*n*	Insulin release (% of insulin content)	
		GLP-1R +/+ islets	*GLP-1R* –/– islets
5.6	5	0.14 ± 0.05	0.05 ± 0.01
8.3	5	0.64 ± 0.14	0.42 ± 0.11
11.1	5	0.97 ± 0.33	0.70 ± 0.20
22.2	5	2.1 ± 0.5	2.0 ± 0.6

Data are means ± SE of five experiments and represent integrated release above basal during the subsequent glucose stimulations of Fig. 5.

TABLE 2
Islet and pancreatic insulin content of control and *GLP-1R*–deficient mice

	Female		Male	
	GLP-1R +/+	*GLP-1R –/–*	*GLP-1R +/+*	*GLP-1R –/–*
Isolated islets (ng/islet)	45.3 ± 5.3 (7)	42.9 ± 4.9 (5)	51.3 ± 7.9 (7)	54.9 ± 9.3 (5)
Total pancreas (µg/g pancreas)	29.4 ± 2.7 (13)	35.8 ± 3.8 (13)	31.9 ± 7.2 (13)	35.1 ± 4.1 (13)

Data are means ± SE (*n*).

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