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GLP-1 Receptors in Golden Syrian Hamster Islets

*Identification and Functional Characterization**

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This study aims at the identification and functional characterization of glucagon-like peptide 1 (7-36) amide (GLP-1) receptor in islets from Golden Syrian hamsters. Using a polyclonal antibody against rat GLP-1 receptors, Western blotting of the islet proteins revealed two major bands of 44 and 70 kDa, similar to those found in rat islets, RINm5F cells, and HIT-T15 cells. In Northern blots, transcripts of 2.7, 3.6 and 3.7 kb were observed in rat islets and RINm5F cells after hybridization with rat GLP-1 receptor cDNA probes of either 219 bp or 1.5 kb. Such was not the case in either hamster islets or HIT-T15 cells. However, a single 3.6-kb transcript was observed in the latter two cases when a human GLP-1 receptor cDNA probe of 1.6 kb was used for hybridization. In the isolated perfused pancreas of Golden Syrian hamsters, a rise in D-glucose concentration from 3.3 to 8.3 m*M* caused a biphasic stimulation of insulin release, which was further increased by either GLP-1 or glucagon (10^{-9} *M* each). The enhancing action of GLP-1 on glucose-stimulated insulin secretion was much more marked than that of glucagon. The rise in D-glucose concentration decreased by 46 ± 4% the release of glucagon, but GLP-1 failed to exert any obvious effect on glucagon secretion in the presence of 8.3 m*M* D-glucose. These results indicate that GLP-1 receptors are expressed in islets of Golden Syrian hamsters with an extracellular part possessing the same immunoreactivity as the rat islet GLP-1 receptors. The expression of the mRNA for the GLP-1 receptor differs, however, from that found in rat or human islets.

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Introduction

The sequence of glucagon-like peptide 1 (7-36) amide (GLP-1), a posttranslational product arising from the tissue-specific processing of preproglucagon, is conserved in mammalian species, including hamster *(1,2)*. The precursor contains, in addition to glucagon or oxyntomodulin *(3)*, the sequences of two glucagon-like peptides, GLP-1 and GLP-2, separated by an intervening sequence. GLP-1 is considered a physiological incretin in humans and other species *(4–8)*. Its insulinotropic effect is dependent on glucose *(6–8)* or nonglucidic nutrient *(9)* concentration, and is currently under investigation for use in the treatment of human noninsulin-dependent diabetes mellitus. GLP-1 mediates its biological effects by binding to a receptor, GLP-1 R, that has been cloned from pancreatic islets in rats *(10)* and humans *(11)*. This receptor is coupled to adenylyl cyclase *(12)* and belongs to the subfamily of heptahelical G-protein-coupled receptors, which were found to regulate B-cell function *(13)*, namely the receptors for glucose-dependent insulinotropic polypeptide *(14)*, glucagon and oxyntomodulin *(15)*, vasoactive intestinal polypeptide and secretin *(16)*, pituitary adenylyl cyclase-activating peptide *(17)*, and calcitonin gene-related peptide *(18)*. The diversification of these molecules during the evolution and the specificity of their cellular expression are important in the definition of their function, and the expression of receptors might vary between species, or between tumoral B-cell lines and normal islet cells. In a previous report, we observed, in hamster tumoral B-cells and HIT cell lines, the presence of both high-affinity glucagon and GLP-1 receptors, selectively coupled to adenylyl cyclase *(19)*. However, receptors of tumoral cells may not retain the characteristics of the corresponding receptors in native cells. In view of the large

Table 1
Characteristics of the Hamsters and Experimental Variables of the Perfusions ($n = 7$)

Hamster weight (g)	143 ± 7
Pancreas wet wt (g)	0.385 ± 0.021
Pancreas insulin content (μg)	60.4 ± 4.8
(μg/g)	157.7 ± 11.2
Pancreas glucagon content (μg)	4.1 ± 0.4
(μg/g)	10.8 ± 1.0
Insulin/glucagon ratio (molar)	8.8 ± 0.5
Flow rate (mL/min)	0.511 ± 0.073
Perfusion pressure (mmHg) min 20	25.5 ± 2.7
min 90	27.4 ± 2.4

use of HIT-T15 cells and the hamster model in diabetes research, it was considered necessary, therefore, to extend our observations to hamster normal islet cells. For this purpose, we have:

1. Verified the presence of GLP-1 R in hamster islets.
2. Examined the effect of GLP-1 on the release of insulin and glucagon from the perfused pancreas.
3. Compared these results to those found in hamster B-cell tumor and in islets of other species.

The experimental results reveal that although the extracellular part of the GLP-1 R in hamster islet cells does not differ immunologically from that in several rodent tumoral cells, the mRNA transcripts of such a receptor are not identical to those in rat or human. Our perfusion data indicate that GLP-1 enhances insulin secretion evoked by glucose to a much larger extent than does glucagon, but fails to affect glucagon release.

Results

Characteristics of the Animals

The characteristics of the animals and perfusion procedure are indicated in Table 1. The insulin content of hamster pancreas was 157.7 ± 11.2 μg/g, and its glucagon content 10.8 ± 1.0 μg/g ($n = 7$ in both cases).

Effects of D-Glucose, GLP-1, and Glucagon on Insulin Release (Fig. 1 and Table 2)

The basal insulin output recorded at 3.3 m*M* D-glucose (min 20–25) and the response to 8.3 m*M* D-glucose (min 27–42) were similar in the GLP-1 and glucagon experiments. The response to the rise in D-glucose concentration was biphasic. GLP-1 caused a further dramatic increase in insulin output (Fig. 1A). Relative to the paired secretory rate recorded in the sole presence of 8.3 m*M* D-glucose, the output of insulin averaged 705 ± 28% ($p < 0.001$) during the 15 min of exposure to GLP-1 (min 42–57) and 1126 ± 47% ($p < 0.001$) when considering only the late period of GLP-1 administration (min 52–57). The release of insulin still represented 1042 ± 68% ($p < 0.001$) of its paired reference value (min 27–42) over the 15-min period following the removal of GLP-1 from the perfusate (min 57–72). Indeed, when the administration of GLP-1 was interrupted at the 55th min of perfusion, the rate of insulin secretion remained elevated for about 5–10 min with an oscillatory pattern comparable to that observed in the sole presence of 8.3 m*M* D-glucose in the present (min 27–42) and prior experiments *(20,21)*.

Glucagon also enhanced glucose-stimulated insulin release (Fig. 1B), but to a lesser extent ($p < 0.001$) than GLP-1. Thus, during the whole period of exposure to glucagon (min 42–57) or during the late part of such a period (min 52–57), the secretory rate averaged only 207 ± 8 and 268 ± 6% ($p < 0.01$), respectively, of the paired reference value (min 27–42). The effect of glucagon, like that of GLP-1, was slowly reversible, the output of insulin between min 57 and 72 still representing 187 ± 14% ($p < 0.025$) of the paired reference value (min 27–42). In both GLP-1 and glucagon experiments, the insulin secretory rate at the end of the experiments (min 80–85) was back to basal value (min 20–25), after that the concentration of D-glucose had been returned to 3.3 m*M* at min 71.

Effects of D-Glucose and GLP-1 on Glucagon Release (Fig. 2 and Table 3)

The basal glucagon output recorded at 3.3 m*M* D-glucose (min 20–25) and GLP-1 experiments averaged 57 ± 8 pg/min, and was reduced by 46 ± 4% ($p < 0.005$) (paired comparison) in response to the rise in hexose concentration to 8.3 m*M* (min 35–40). The administration of GLP-1 exerted no obvious effect on the low glucagon secretory rate prevailing in the presence of 8.3 m*M* D-glucose (Table 3). The late return to the initial low concentration of D-glucose (3.3 m*M*) reversed the hexose-induced inhibition of glucagon release. Indeed, although the mean glucagon output recorded in the presence of 8.3 m*M* D-glucose decreased by 4.3 ± 1.6 pg/min between min 41–55 and min 56–70, an opposite change was observed thereafter (min 75–85), the difference between the decrement and increment in secretory rate recorded, respectively before and after the lowering of D-glucose concentration averaging 15.7 ± 8.8 pg/min. Moreover, whereas the mean glucagon output recorded in the presence of 8.3 m*M* D-glucose between min 56 and 70 averaged 37.5 ± 11.5% ($p < 0.05$) of the paired initial value found at 3.3 m*M* D-glucose, no significant difference was observed when comparing such an initial value to that found at the end of the experiments (min 75–85).

Characterization of GLP-1 in Rat and Hamster Islets: Western Blots

A representative autoradiogram of the proteins that were detected by polyclonal antibody against rat GLP-1 R is shown in Fig. 3. The immunolocalization of GLP-1 in hamster islets as two bands of 44 and 70 kDa is similar to that

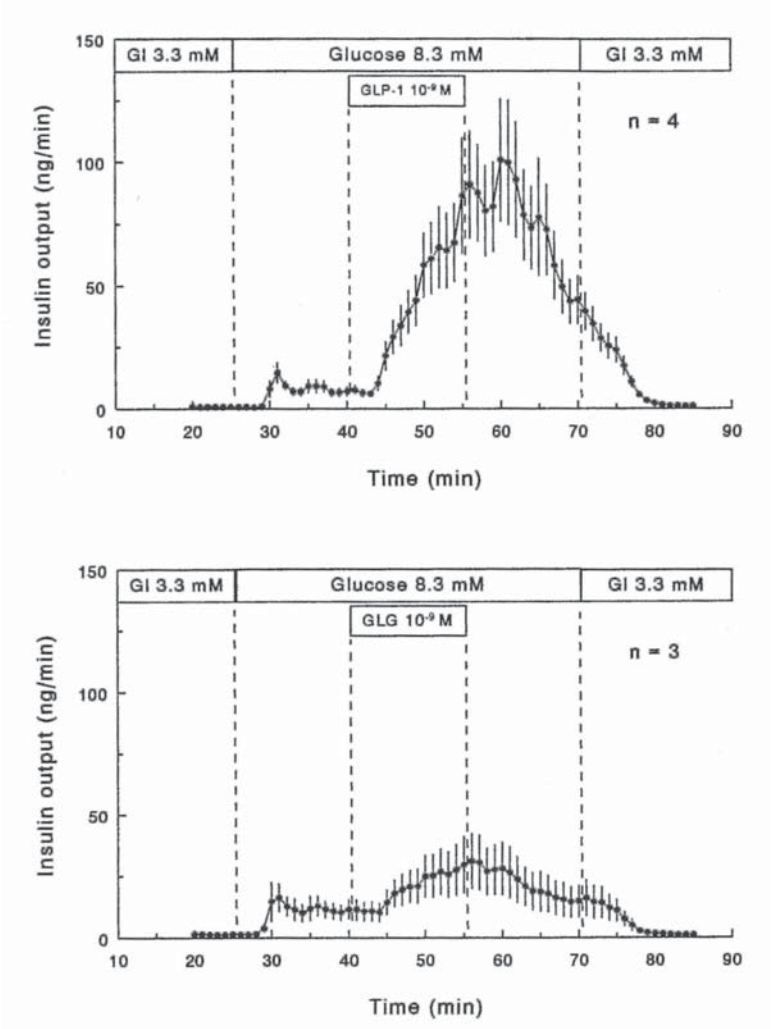

Fig. 1. Effect of 8.3 m*M* D-glucose (**A** and **B**) and 10^{-9} *M* GLP-1 (A) or glucagon (GLG; B) on the secretion of insulin from the perfused pancreas of Golden Syrian hamsters. Mean values (±SEM) are shown together with the number of experiments (*n*).

Table 2
Insulin Secretory Rates (ng/min) from the In Vitro Perfused Hamster Pancreas at Basal (3.3 m*M*) or Elevated (8.3 m*M*) Glucose and During Administration at 10^{-9} *M* GLP-1 (Fig. 1A) or Glucagon (Fig. 1B)

		GLP-1 experiments, ($n = 4$)	Glucagon experiments, ($n = 3$)
Glucose 3.3 m*M*	(min 20–25)	0.8 ± 0.3	1.3 ± 0.8
Glucose 8.3 m*M*	(min 27–42)	7.1 ± 2.0	10.5 ± 3.8
Glucose 8.3 m*M* + GLP-1 or glucagon	(min 42–57)	48.4 ± 11.7	21.7 ± 7.7
Glucose 8.3 m*M* + GLP-1 or glucagon	(min 52–57)	77.3 ± 18.9	28.5 ± 10.6
Glucose 8.3 m*M*	(min 57–72)	70.5 ± 16.7	20.6 ± 8.3
Glucose 8.3 m*M*	(min 80–85)	1.3 ± 0.3	1.3 ± 0.9

found in rat islets. Addition of the peptide corresponding to the extracellular domain of the receptor completely suppressed these two bands in different B-cell preparations, including HIT-T15 and RINm5F cells (data not shown). These bands correspond to those found in purified rat B-cells *(22)*, and were also found in RINm5F and HIT-T15 cells (Fig. 3, lanes 2 and 4). No labeled protein was detected in rat or hamster pancreas homogenates (Fig. 3, lanes 5 and 6), owing to the dilution of islet proteins that do not exceed 2% of the total pancreatic protein content. It was indeed already shown that there is no GLP-1 R in rat or guinea pig acinar cells *(23)*. The 134-kDa band present in islets was also detected in fibroblasts BP-A31 (Fig. 3, lane 8) used as negative control and, thus, represents a

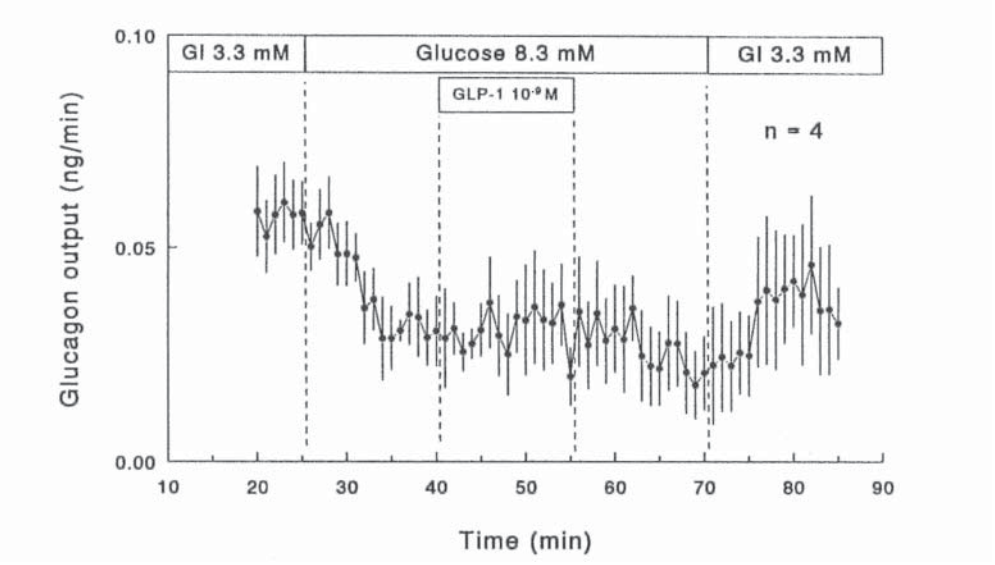

Fig. 2. Effect of 8.3 m*M* D-glucose and 10^{-9} *M* GLP-1 on the secretion of glucagon from the perfused pancreas of Golden Syrian hamsters. Mean values (±SEM) are shown together with the number of experiments (*n*). Experiments are the same as those illustrated in the upper panel of Fig. 1.

Table 3
Glucagon Secretory Rates (pg/min) from the In Vitro Perfused Hamster Pancreas at Basal (3.3 m*M*) or Elevated (8.3 m*M*) Glucose Concentrations and During Administration of 10^{-9} *M* GLP-1[a]

Glucose 3.3 m*M*	(min 20–25)	57 ± 8
Glucose 8.3 m*M* (pre-GLP-1)	(min 35–40)	31 ± 6
Glucose 8.3 m*M* + GLP-1	(min 41–55)	31 ± 8
Glucose 8.3 m*M* (post-GLP-1)	(min 56–70)	27 ± 9
Glucose 3.3 m*M*	(min 75–85)	38 ± 14

[a]The results correspond to the experiments illustrated in Fig. 2 (*n* = 4).

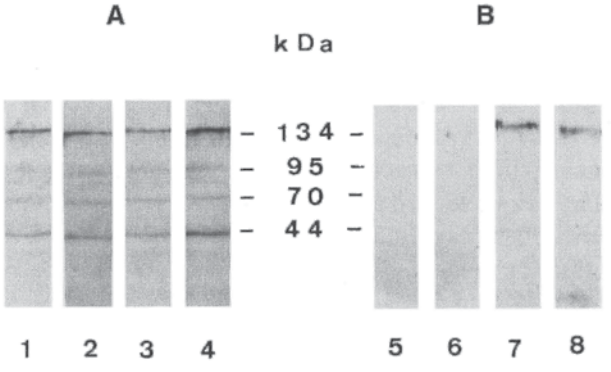

Fig. 3. Western blot analysis. Representative autoradiogram of various proteins labeled by a rat GLP-1 R polyclonal antibody (1:500 dilution). Tissue and cell homogenate proteins were separated by SDS-PAGE and transferred to nitrocellulose for immunoblotting. Immunoreactive proteins were made visible using chemiluminescence technique and autoradiography (*see* Materials and Methods for details).**(A)** rat (lane 1) and hamster (lane 3) islets (125 µg protein/lane); RINm5F (lane 2) and HIT-T15 (lane 4) cells (125 µg protein/lane). **(B)** rat (lane 5) and hamster (lane 6) pancreas (150 µg protein/lane); rat intestinal cryptic cells (lane 7) and mouse BP-A31 fibroblasts (lane 8) (170 µg protein/lane).

crossreacting protein not related to the GLP-1 R. In rat and hamster islet extracts, there were two unidentified bands at 80 and 95 kDa, which were more marked in transformed cells. A very faint band was seen at 44 kDa in intestinal cryptic cells (Fig. 3, lane 7).

Characterization of GLP-1 R in Rat and Hamster Islets: Northern Blots

After 16 h of exposure, a strong signal was observed in rat islets and in RINm5F cells used as positive controls (Fig. 4). Transcripts of 2.7, 3.6, and 3.7 kb were observed after hybridization with rat GLP-1 R cDNA probes of either 219 bp (Fig. 4A) or 1.5 kb in rat islets and RINm5F cells (data not shown). They are in agreement with those obtained with the same probe in the calcitonin- and CGRP-secreting rat C-cell line that was shown to possess a GLP-1 R *(24)*. No GLP-1 R mRNA transcript was detected with total RNA of hamster islets or HIT-T15 cells using either the 219-bp rat probe or rat and human probes covering the entire coding sequence of the receptor. When the human cDNA probe of GLP-1 R was used, a weak signal for a single 3.6-kb mRNA transcript was observed in low-stringency condition (2X SSC followed by 1% SDS for 30 min at 45°C) with total RNA of hamster islets and HIT-T15 cells (Fig. 4B). This signal corresponds to the 3.6-kb transcript observed in rats. Other bands observed at low stringency are nonspecific.

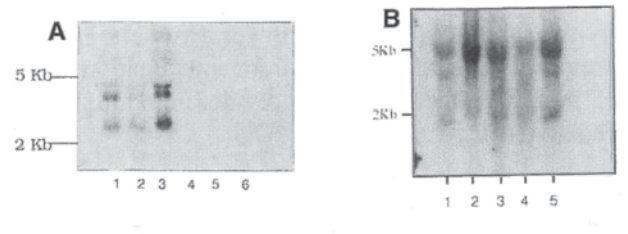

Fig. 4. Northern blot analysis of GLP-1 R. **(A)** total RNA from rat islets (lane 1, 20 µg/lane), RINm5F cells (lane 2, 20 µg/lane; lane 3, 40 µg/lane), hamster islets (lane 4, 20 µg/lane), and HIT-T15 cells (lanes 5 and 6, 20 µg/lane) was hybridized using a rat GLP-1 R cDNA probe of 219 bp. **(B)** total RNA from RINm5F cells (lane 1, 20 µg/lane), rat islets (lane 2, 20 µg/lane), hamster islets (lane 3, 20 µg/lane), and HIT-T15 cells (lanes 4 and 5, 20 µg/lane) was hybridized using a human GLP-1 R cDNA probe of 1.6 kb.

Discussion

Our results extend previous studies of the response to GLP-1 and glucagon conducted in a Syrian hamster insulinoma and the HIT-T15 cell line to the islet cells of normal Golden Syrian hamsters *(19,25)*, and provide new information on the effect of GLP-1 on insulin and glucagon secretion in this species.

Using a polyclonal antibody directed against the entire extracellular domain of the rat GLP-1 R sequence, two major proteins of ~44 and ~70 kDa were identified in hamster islets and HIT-T15 cells like in rat islets and RINm5F cells. This indicates the presence of a very similar immunological determinant in hamster and rat GLP-1 R. The ~70 kDa value is in the range of the ~65 kDa found for the plasma membrane protein covalently linked to [^{125}I]GLP-1 in hamster insulinoma or HIT-T15 cells *(19,25)*. It may thus correspond to the mature form of the receptor. The ~45 kDa form probably corresponds to the immature, nonglycosylated form of the receptor *(26)*. Both bands were suppressed by the peptide corresponding to the extracellular domain of GLP-1 R. The molecular weight of GLP-1 R was found to vary between 53 and 67 kDa in distinct tissues and animal species *(22,27)*. These variations might be explained, at least for those GLP-1 R that have the same deduced sequence *(28)*, by a specificity in the posttranslational process leading to the glycosylation of GLP-1 R *(26)*, which can take place on three potential N-linked glycosylation sites in rat and humans *(10,11)*. On the other hand, the sequences of all the biologically active GLP-1 binding sites *(29)* are not known, and the existence of structural variants of the GLP-1 R is likely *(30)*. The hamster GLP-1 R is not observed in Northern RNA when rat probes are used and only detected in condition of low stringency with a human probe. It is not surprising that the small rat probe gave no signal, since it is directed toward specific transmembrane stretches less likely to be conserved between species. Although some analogy is suggested by the experiments in low-stringency condition, the main transcript of 2.6 kb and the associated transcripts of 4.1, 5.0, and 6.0 kb observed in humans *(11)* were not present in hamster islets. This indicates a species difference of the hamster cDNA sequence that does not seem to modify the main properties of GLP-1 R.

In normal Golden Syrian hamsters, the islet GLP-1 R displays the same responsiveness to GLP-1 as in rats, mice, or humans, as documented by the efficient enhancement of glucose-stimulated insulin release. The insulinotropic action of GLP-1 is currently attributed to a sequence of events involving the binding of the peptide to its receptor and the subsequent stimulation of adenylyl cyclase. When GLP-1 is removed, the reversibility of these events is ensured by the internalization of receptor-bound ligand molecules and the phosphodiesterase-catalyzed conversion of cAMP to 5'-AMP.

In the hamsters, like in other species, the insulinotropic potency of GLP-1 largely exceeded that of glucagon *(6,31,32)*. There is a good correlation between the efficiency of these peptides in stimulating insulin release and activating adenylyl cyclase *(12)*. The ED_{50} for stimulation of insulin release, as well as that for activation of adenylyl cyclase, is one to two orders of magnitude lower with GLP-1 than glucagon, whereas maximally active doses of these peptides might result in similar values for insulin secretion or adenylyl cyclase activity *(19,22,32)*. The prevalence of GLP-1 over glucagon in stimulating insulin release probably resides, therefore, mainly in the process coupling the receptor occupancy to the activation of adenylyl cyclase. It might be hypothesized that the GLP-1 and the glucagon receptors are differently coupled in islet B- and A_2-cells, with the possible participation of different α-subunits of stimulatory G-proteins *(33)* or different isoforms of adenylyl cyclase *(34)*.

The presence of GLP-1 R on the glucagon-secreting A_2-cells cannot be ascertained from our experiments. In the present study, there was no effect of GLP-1 on glucagon release, in agreement with some prior observations *(6,9,35,36)*. In other studies, however, GLP-1 was found either to inhibit glucagon release *(5,7,31,32,37,38)* or to increase exocytosis in purified rat A_2-cells *(39)*. Likewise, cAMP or agents increasing the cell content in cAMP were found either to enhance or inhibit glucagon secretion *(40–44)*. The presence *(45,46)* or absence *(22)* of GLP-1 R on glucagon-producing cells also represents a controversial matter.

In order to reconcile some of these observations, it could be postulated that such binding sites belong to a subtype of GLP-1 R not coupled to adenylyl cyclase. Another hypothesis could be that when observed, the decrease in glucagon output caused by GLP-1 is owing to the suppressing effect of insulin and/or somatostatin in a paracrine process *(39)*. Last, the most naive explanation for the present findings would consist of the fact that in the presence of 8.3 m*M* D-glucose, the inhibitory effect of the hexose on glucagon release impedes either a further decrease in secretory rate

or a possible enhancing action of GLP-1 on A_2-cell secretory activity.

In the present study, we have thus demonstrated that GLP-1 receptors are expressed in islets from normal Golden Syrian hamsters, with an extracellular part possessing the same immunoreactivity like that of the rat islet GLP-1 R. The mRNA for the GLP-1 R in the hamster islets differs, however, from that in rat or human islets. Activation of the hamster receptor by GLP-1 results in a marked potentiation of glucose-induced insulin release, largely exceeding that caused by glucagon. In the presence of 8.3 m*M* D-glucose, GLP-1 does not affect glucagon release by the hamster pancreas. As already mentioned, cell differences in GLP-1 action may depend, *inter alia*, on the G-protein-stimulatory subtypes ($G\alpha_s$ and $G\alpha_{olf}$) and adenylyl cyclase isoforms (I–VIII; *see* ref. *47*), which appear differently expressed in B- and A_2-cells *(33,34)*. It depends also on the nature of the synergistic action between agonists acting on G_s-coupled receptors and those increasing intracellular concentration of Ca^{2+} *(48–50)*. Further investigations are required to assess whether the differences observed here in the nonextracellular coding part of the hamster and rat islet GLP-1 R mRNA might affect the functional response to GLP-1 of B- and non-B-islet cells.

Materials and Methods

Synthetic human GLP-1 (7-36) amide was obtained from Peninsula Laboratories (St. Helens, Merseyside, UK) and porcine glucagon from Sigma (St. Louis, MO). A polyclonal antibody against S-transferase was raised in rabbit by S. Mojsov (Rockefeller University, New York, NY) against a fusion protein consisting of the entire extracellular domain (up to the first membrane-spanning domain) of the rat GLP-1 R sequence and glutathione S-transferase.

Pancreatic Islet and Cell Line Preparation

Adult female Golden Syrian hamsters (*Mesocricetus auratus*, 115-165 g) were obtained from Charles River Laboratory (Sulzfeld, Germany) and Wistar rats (200–250 g) from Proefdierencentrum (Heverlee, Belgium). They were housed under constant conditions of lighting and temperature with free access to food and water, until the experiment. Hamster and rat islets were isolated from the pancreas by a collagenase (type P, Boehringer, Penzderg, Germany) digestion technique *(51)*. Islets were manually picked under a stereomicroscope, collected, and washed in chilled Hank's buffer. The B-cell lines used were HIT-T15 cells derived from hamster islet cells transformed by SV-40 *(52)* and RINm5F cells, a continuous, clonal, insulin-secreting cell line established from a transplantable rat islet cell tumor known to possess GLP-1 R *(53)*. Intestinal cryptic cell line (rIC) and mouse fibroblasts (BP-A31) were used as controls. Islets and cells were homogenized in lysis buffer (pH 7.5) in the presence of protease inhibitors, 1 m*M* phenylmethylsulfonylfluoride, and 0.1 mg/mL of aprotinin as trypsin inhibitor (Trasylol®; Bayer, Brussels, Belgium) as previously described *(54)*. After centrifugation at 600*g* for 5 min, the supernatant was divided into aliquots and stored at –80°C.

Immunoblot Analyses

The protein content of the stored samples was measured *(55)* and the presence of GLP-1 R was assessed by Western blotting as described elsewhere *(56)*. In brief, pancreatic islet and cell proteins were separated by SDS-polyacrylamide gel (12%) and transferred onto a nitrocellulose membrane (Hybond ECL; Amersham, Buckhinghamshire, UK). The membranes were blocked overnight by a buffer containing 10% defatted skimmed milk. They were then incubated for 4 h at room temperature with anti-GLP-1 R antibody. The labeled proteins were revealed by a species-specific second antibody, the antirabbit Ig horseradish peroxidase-linked F(ab') 2 fragment from donkey (NA9340). After extensive washing, the membrane was bathed in a chemiluminescent reagent ECL (Amersham) and visualized by exposure to Kodak Biomax MR for 30 s. Molecular weights of labeled proteins were determined using prestained mol-wt markers (Bio-Rad, Hercules, CA).

RNA Extraction and Northern Blot Analysis

Total RNA was extracted from rat and hamster islets, and HIT-T15 and RINm5F cells using the guanidium thiocyanate method *(57)*. Twenty micrograms of total RNA were denatured by formaldehyde, subjected to electrophoresis on 1% agarose gel and transferred to gene screen plus membranes (NEN, Research Products, Boston, MA). The filters were prehybridized in buffer containing 50% formamide, 5X SSC (1X SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate), 5X Denhardt solution, and 0.1% sodium dodecyl sulfate (SDS) and sonicated herring sperm DNA (100 µg/mL). Hybridization was carried out overnight at 42°C in the same buffer containing a rat GLP-1 cDNA probe of 219 bp specific of transmembrane stretches IV and V *(24)*, a rat GLP-1 R cDNA of 1.5 kb, or a human GLP-1 R cDNA of 1.6 kb *(28)* labeled by nick translation using [α-^{32}P]dCTP (Amersham). The latter two probes cover the entire coding sequence of the receptor. Filters were washed twice at room temperature in 2X SSC for 5 min, twice in 2X SSC + 1% SDS for 30 min at 55°C, and twice in 0.1X SSC for 30 min at room temperature. The washed filters were exposed overnight at –80°C to X-OMAT™ AR 5 films (Biomax™ MS; Eastman Kodak, Rochester, NY).

Perfusion Procedure

The perfusion procedure was inspired in several respects by previous publications *(58,59)*. The hamsters were anesthetized with sodium pentobarbital in the range of 42–56 mg/kg ip, and anesthesia was maintained further with ether. The surgical procedure proved to be technically

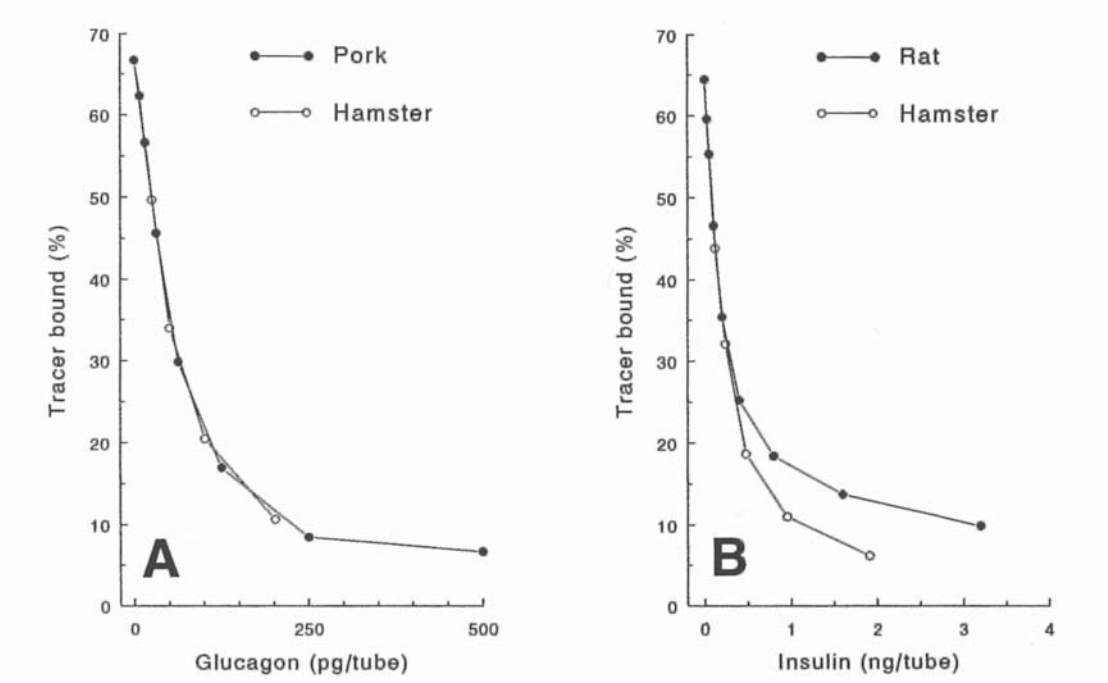

Fig. 5. (A) Binding of porcine ^{125}I-glucagon by rabbit antiglucagon serum in the presence of either increasing concentrations of a porcine glucagon standard or serial dilutions of a hamster pancreas extract. **(B)** Binding of porcine ^{125}I-insulin by guinea-pig anti-insulin serum in the presence of increasing concentrations of either a rat insulin standard or serial dilutions of a hamster pancreas extract.

difficult, mainly because the celiac artery originated above the diaphragm, and from a deeply located aorta. The pancreas was perfused through the celiac and superior mesenteric arteries as previously described in rat *(51)*. After an equilibration period of 20 min, samples of the pancreatic effluent were collected from the portal vein at 1-min intervals in chilled tubes containing 1000 kallikrein inhibitor units of aprotinin (Trasylol; Bayer, Brussels, Belgium) and 1.2 mg of EDTA. The basal perfusion medium contained D-glucose (3.3 m*M*). The concentration of D-glucose was raised to 8.3 m*M* from min 26 to 70. From min 41 to 55, either human GLP-1 or a mixture of bovine and porcine glucagon (Sigma) was administered, in order to reach a final concentration of 10^{-9} *M* in the perfusate, through a sidearm syringe working at a flow rate of 0.075 mL/min (Unita I infusion pump, Braun Melsungen, FRG). Both the GLP-1 and glucagon solutions were prepared freshly 10 min prior to administration in saline supplemented with aprotinin (500 kallikrein inhibitor U/mL) and bovine serum albumin (5 mg/mL, fraction V, RIA grade; Sigma). The perfusion pressure was recorded with a blood pressure monitor (Palmer, London, UK). At the end of the perfusion, the pancreas was weighed and extracted using acidified ethanol *(51)*. The effluent samples and pancreas extracts were stored at –25°C until assay.

Radioimmunoassay (RIA)

Insulin and glucagon RIA were performed in the pancreas extracts and in the effluent samples as previously described *(60)*. There was no interference of GLP-1 or glucagon in the insulin RIA. Serially diluted extracts of Golden Syrian hamster pancreas behaved identically to rat insulin (Novo Nordisk, Bagsvaerd, Denmark) in the low range (0.025–0.2 ng insulin) of the standards (Fig. 5). Differences were only observed at higher concentrations, hamster insulin being more efficient than rat insulin in competing with porcine ^{125}I-insulin for the polyclonal guinea pig antibodies raised against beef-pork insulin. This may be related to difference in the structure of insulin in these two species *(61)*. The insulin content of the pancreatic effluent and extracts was estimated, therefore, by reference to the serially diluted extracts of hamster pancreas only in the high-affinity domain of the assay. In the glucagon assay, the serially diluted extracts of the hamster pancreas behaved identically to the pork glucagon (Sigma) used as standard throughout the range of the concentrations tested (0–500 pg of pork glucagon/tube). The integrated insulin or glucagon output was computed from the area under the curves. All results are presented as mean values ± SEM, together with the number of individual determinations (*n*). The statistical significance of differences between mean values was assessed by Student's two-tailed *t*-test for unpaired data.

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