Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 330 (2005) 327–332
BBRC
www.elsevier.com/locate/ybbrc

# Glucagon production of the rat insulinoma cell line INS-1—A quantitative comparison with primary rat pancreatic islets ☆

L. Cornelius Bollheimer *, Christian E. Wrede, Felix Rockmann, Iris Ottinger, Jürgen Schölmerich, Roland Buettner

*Department of Internal Medicine I, University of Regensburg, 93042 Regensburg, Germany*

Received 10 December 2004

**Abstract**

The rat insulinoma cell line INS-1 is the most commonly used clonal cell model in pancreatic β-cell research. Considering the multihormonality of many insulinomas we examined as to how INS-1 cells comply with the notion of resembling a pure β-cell line. Glucagon immunoassays revealed that INS-1 cells secrete glucagon in a similar range as islets. By immunohistochemistry we detected a cytoplasmic glucagon signal in INS-1 cells which colocalized with C-peptide. Cellular content of preproglucagon-mRNA and glucagon protein in INS-1 cells was less than two percent of the respective values in islets, which probably reflects differences in the intracellular metabolism and/or secretory pathways. Taken together, it is obvious that INS-1 cells do not represent an exclusively insulin producing β-cell line.


*Keywords:* INS-1; Insulinoma; Islet; Glucagon; Preproglucagon-mRNA; Free fatty acids

The insulin-secreting cell line INS-1 was established in 1992 by dispersion of a radiation-induced insulinoma from NEDH rats into a tissue culture medium containing β-mercaptoethanol [1]. Since INS-1 cells secrete insulin in response to glucose (GSIS) and maintain a satisfying degree of differentiation, they are generally considered to be a less laborious alternative to isolated islets of Langerhans and have been employed as β-cell-model in nearly five hundred studies so far. An important drawback to the INS-1 cell line, however, is its polyclonal nature which is reflected by the presence of glucose-responsive and glucose-unresponsive subpopulations [2]. To circumvent this problem, subcloning with or without additional genetic manipulation has led to clonal INS-1-derived cell lines [3–5], but these are not as widely used as the parental INS-1 cells.

A second general problem of insulinoma-derived cells, namely that of multihormonality, has received only little attention in INS-1 cells so far. From human pathology it is known that nearly fifty percent of all insulinomas are plurihormonal tumors which secrete not only insulin but also glucagon and other islet hormones [6–8]. Parental INS-1 cells are generally regarded as a non-glucagon-secreting cell line [1,9]. However, this notion is mainly based on the lack of detection of preproglucagon-mRNA in Northern blot analysis, notwithstanding the fact that the more sensitive reverse transcription PCR could trace mRNA of all four islet hormones in INS-1 cells [9]. mRNA abundance does not necessarily reflect protein expression, but up to now, glucagon *secretion* has not been investigated in INS-1 cells.

In view of the close, glucose-dependent paracrine interaction between α-cellular and β-cellular function [10,11], it is important to know whether the glucagon

☆ *Abbreviations:* IRI, immunoreactive insulin; PPI, preproinsulin; PPG, preproglucagon.

* Corresponding author. Fax: +941 944 7011.
*E-mail address:* cornelius.bollheimer@klinik.uni-regensburg.de (L.C. Bollheimer).

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.01.168

protein itself is produced by a cell line widely used as model for the analysis of insulin secretion. Therefore, we systematically analyzed different parameters of α-cell function in INS-1 cells and characterized them quantitatively by a direct comparison with primary rat pancreatic islets.

## Materials and methods

*Reagents*. Radioimmunoassays for rat pancreatic glucagon (cross-reactivity to oxyntomodulin <0.1%) and for immunoreactive insulin (IRI) were purchased from Linco Research (St. Louis, USA). For immunofluorescence, the antiserum (derived from guinea-pig) against rat c-peptide was from Linco Research (St. Louis, USA), the specific monoclonal antibody (derived from mouse) against rat glucagon was from Sigma–Aldrich (Taufkirchen, Germany), the secondary guinea-pig immunoglobulin antibody conjugated to red Cy3 was from Jackson ImmunoResearch (West Grove, USA), and the secondary mouse immunoglobulin antibody conjugated with Oregon green was from Molecular Probes (Eugene, USA). Technical and chemical equipment for semiquantitative Light Cycler PCR were from Roche Diagnostics (Mannheim, Germany). Reverse transcription was performed employing a kit from Promega (Madison, USA). All other analytical-grade biochemicals were purchased either from Sigma–Aldrich (Taufkirchen, Germany) or from Merck Eurolab (Darmstadt, Germany).

*Handling of INS-1 cells and primary rat pancretic islets*. INS-1 cells (for passages see below) were cultivated in RPMI-1640/11.2 mM glucose/10% fetal calf serum containing 2 mmol $L^{-1}$ L-glutamine 1 mmol $L^{-1}$ sodium pyruvate, 100 U $mL^{-1}$ penicillin, 100 μg $mL^{-1}$ streptomycin, and 50 μmol $L^{-1}$ β-mercaptoethanol at 37 °C in a 5% $CO_2$ atmosphere [1]. Unless otherwise stated, the cells were sub-cultured or used for the experiments at a confluence of about 80%. Pancreatic islets of Langerhans were isolated from male 200 g Wistar rats as described previously [12] and were employed for the experiments after hyperglycemic preconditioning for 16 h in RPMI-1640/11.2 mM glucose/10% fetal calf serum at 37 °C in a 5% $CO_2$ atmosphere.

*Glucagon and insulin release in the presence of glucose and/or free fatty acids*. INS-1 cells cultured at 11.2 mM glucose (passage #45–#50) were washed and incubated at 37 °C in Krebs–Ringer buffer containing 16 mM Hepes (pH 7.4) and 0.1% fatty acid ultra-free BSA. The starting glucose concentration was 1.4 mM. The supernatant was removed after 30 min of incubation and substituted by fresh medium containing two times the amount of glucose. This was precisely repeated over 150 min up to a final glucose concentration of 22.4 mM. Corresponding studies were also conducted with batches of 20 primary pancreatic islets each. In another set of experiments, INS-1 cells were incubated in Krebs–Ringer buffer containing 5.6 mM glucose with or without 200 μM palmitate (precomplexed to 0.2% BSA) for 2 h. Immunoreactive insulin and glucagon were determined from the supernatants and the cellular lysates were harvested at the end of the experiments. The values were normalized by the total cellular protein as determined by the bicinchoninic method.

*Light cycler analysis of cellular preproglucagon- and preproinsulin-mRNA*. Total RNA was prepared from different passages of INS-1 cells (#51–#76) and from primary pancreatic islets using Qiagen RNeasy kits. First strand complementary DNA was synthesized by priming with random hexamers. Subsequent real-time polymerase chain reaction for preproglucagon-mRNA (PPG-mRNA), preproinsulin-mRNA (PPI-mRNA), and 18S-rRNA was performed as previously described [12].

*Immunofluorescent detection of cellular glucagon and insulin*. INS-1 cells were cultured on glass slides (Nunc Lab-Tek, Wiesbaden, Germany) until 50–60% confluence. The cells were washed with ice-cold PBS, fixed with 4% formaldehyde in PBS, and permeabilized with 0.5% saponin in PBS for 15 min. The fixed cells were rinsed with PBS and pre-incubated with 1% donkey serum in PBS for 30 min, followed by incubation with primary antiserum/antibody against C-peptide and glucagon at a 1:300 dilution in 2% BSA/PBS at 4 °C overnight. The cells were washed three times with PBS and incubated with a secondary antibody conjugated to either Oregon green or Cy3 at a dilution of 1:300 in 2% BSA/PBS at 25 °C for 1 h. The glass slides were washed and dried, coverslips were mounted with media. Slides were examined with a Leica DM RBE fluorescence microscope (Leica Microsystems, Wetzlar, Germany).

*Statistical analysis*. Data are presented as means ± standard deviation or as Tukey's box-blot. Statistically significant differences were analyzed using an exact Fisher–Pitman–Permutation Test (for $n \leqslant 5$) or an unpaired Student's $t$ test (for $n > 5$). The significance level was set to $P \leqslant 0.05$.

## Results and discussion

### *INS-1 cells release both insulin and glucagon in relevant amounts*

The insulin and glucagon release of INS-1 cells ($n = 5$ experiments, each containing four independent samples in parallel) and rat pancreatic islets ($n = 3$ experiments, each containing four independent samples in parallel) was monitored over 150 min with glucose concentrations increasing stepwise from 1.4 to 22.4 mM. The secretion values for immunoreactive insulin and glucagon were normalized by the cellular protein content.

Before exploring a putative α-cellular behavior of the INS-1 cells, we first validated our model in terms of the known β-cellular feature. In accordance with previous findings [1,2] the release of immunoreactive insulin (IRI) in INS-1 cells was stimulated by twofold as the glucose concentration was stepwise raised from 1.4 to 22.4 mmol $L^{-1}$ ($P = 0.004$, Fig. 1A). The secretory rate ranged between 250 and 750 fmol $mg^{-1}$ protein $min^{-1}$ which is in the same order of magnitude as previously described [13]. Primary rat pancreatic islets which had been pre-conditioned at 11.2 mM (see Materials and methods) showed an average sevenfold increase of IRI secretion during the glucose challenge ($P = 0.05$; Fig. 1A). The cumulative IRI-secretion from INS-1 cells during the 150-min glucose challenge equalled that from rat pancreatic islets (100 ± 37%; Fig. 2A) whereas the intracellular IRI content was halved (49 ± 16%, $P = 0.018$; Fig. 2B). In terms of secretion, one has to note that the IRI output of INS-1 cells was in tendency higher at low glucose concentrations but lower at high glucose concentrations when compared with rat pancreatic islets (Fig. 1A). This secretory profile is compatible with a left shift in glucose sensitivity and a blunted dose–response curve for INS-1 cells as it has been demonstrated for other clonal β-cell models [14].

Having demonstrated a proper *insulin* secretory function in the INS 1-cells, we next examined whether the *same* cells also show a relevant *glucagon* production

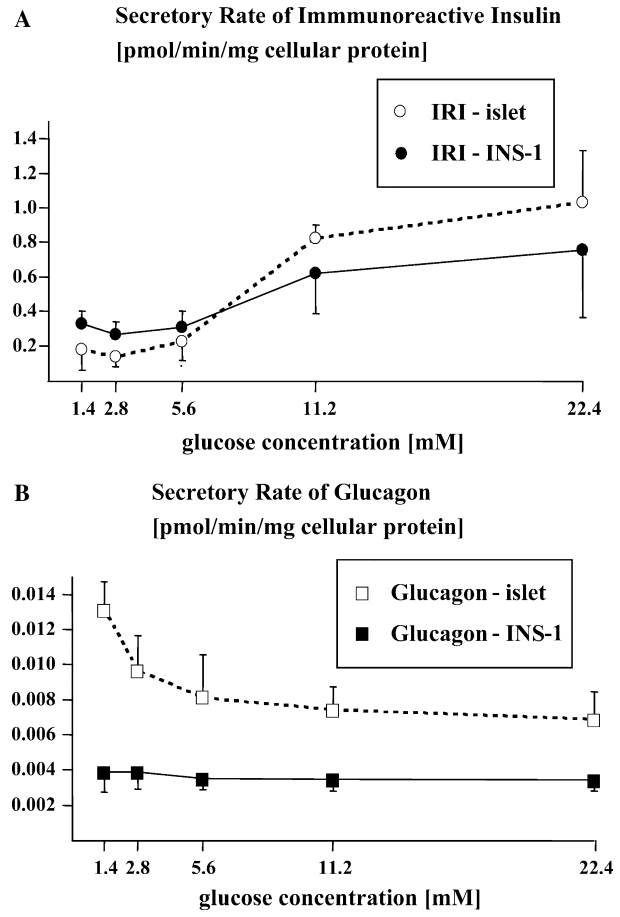


Fig. 1. Glucose-dependent release of insulin and glucagon in INS-1 cells compared with rat pancreatic islets. INS-1 cells and primary rat pancreatic islets were preconditioned at 11.2 mM glucose and subsequently exposed to steadily increasing glucose concentrations. Starting with 1.4 mM glucose in the incubation buffer, the cellular supernatant was replaced every 30 min by a fresh one containing the double amount of glucose (i.e., 1.4, 2.8, 5.6, 11.2, and 22.4 mM glucose). Secretion rates for immunoreactive insulin (A) and glucagon (B) are depicted (means ± standard deviation). Normalization by cellular protein allows a quantitative comparison between INS-1 cells and primary rat pancreatic islets.

and release. During the glucose challenge (as described above) we found a stable glucagon secretory rate in INS-1 cells of $3.6 \pm 0.1$ fmol $mg^{-1}$ $min^{-1}$. In a direct comparison, rat pancreatic islets revealed a clear glucose-dependency on glucagon secretion (Fig. 1B). Here, the maximum secretion rate of glucagon ($13.1 \pm 1.6$ fmol $mg^{-1}$ $min^{-1}$) was found immediately after the initial transfer of the islets from hyperglycemic culture medium (11.2 mM glucose) into the hypoglycemic incubation medium (1.4 mM glucose). Later, the secretory rates for glucagon in pancreatic islets fell markedly and reached a minimum at 22.4 mM glucose ($6.9 \pm 1.6$ fmol $mg^{-1}$ $min^{-1}$, $P = 0.05$ versus the secretory rate at 1.4 mM glucose). These latter findings suggest a glucose dependency of islet glucagon secretion to some degree, but they also would agree with the so-called switch-off hypothesis which proposes the sudden drop of extracellular insulin to be an important glucagonotropic cofactor for isolated pancreatic islets at low glucose levels [11,15,16]. The cumulative secretion of glucagon from INS-1 cells amounted to $39 \pm 7\%$ of that from pancreatic islets ($P = 0.018$; Fig. 2C) whereas intracellular glucagon content was markedly lower in INS-1 cells ($1.2 \pm 0.5\%$ of the islet glucagon content, $P = 0.018$; Fig. 2D). Taken together, notwithstanding the small intracellular glucagon stores, these comparative data prove a quantitatively relevant glucagon release from INS-1 cells for the first time.

### *INS-1 cells produce both glucagon and insulin*

The glucagon secretion observed above could (i) be caused by a specialized subclone of the polyclonal INS-1 cells or (ii) be due to a plurihormonal feature of these cells in general. To further clarify this issue, we next performed immunohistochemistry for both C-peptide (as a surrogate marker of insulin) and glucagon. Here, the presence of glucagon and C-peptide could be shown in the same cells arguing for a bihormonal feature of INS-1 cells (Fig. 3).

### *Palmitate elicits both insulin and glucagon secretion in INS-1-cells*

In rat pancreatic islets, we and others have very recently described that free fatty acids (FFA) stimulate not only insulin secretion but act also as a specific secretagogue for glucagon in the α-cell [17,18]. When monitoring INS-1 cells for insulin release in the presence or absence of 200 μM palmitate ($n = 3$ experiments, each containing 4 independent samples in parallel), we found a clear stimulatory effect of fatty acids with a 3.8 (± 1.3) fold increase ($P = 0.05$) as described before [19]. In addition, there was also a fatty acid-induced increase in glucagon secretion with a 3.2 (±0.6) higher glucagon release ($P = 0.05$).

From studies on primary pancreatic islets and INS-1 cells there is abundant evidence that acute exposure to FFA increases the β-cellular insulin secretion due to the essential role of fatty acid derivatives within the metabolic stimulus response coupling (for detailed review see [20]). However, even in primary pancreatic islets the regulation of α-cellular glucagon secretion is not completely understood. Next to paracrine effects [21–23], intermediates of glycolysis or fatty acid β-oxidation may also represent metabolic signaling molecules for α-cellular glucagon secretion [17,22]. Based on the latter perception and our present data one might speculate that FFA and/or metabolites might interfere with a common mechanism of insulin and glucagon release. Nonetheless, this issue certainly requires further investigation.

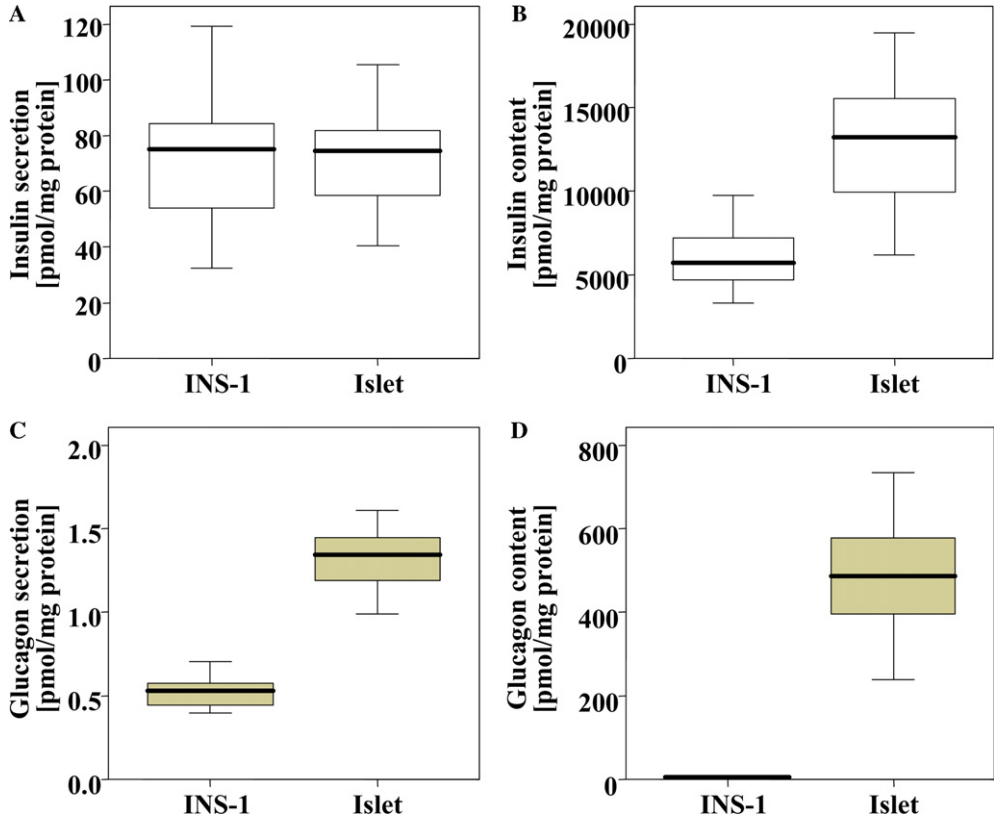


Fig. 2. Cumulative release and final intracellular content of insulin and glucagon in INS-1 cells and rat pancreatic islets. INS-1 cells and rat pancreatic islets were treated as described in Fig. 1. The cumulative secretion of insulin (A) and glucagon (C), and the cellular content of insulin (B) and glucagon (D) are depicted as Tukey's box plots. Data are derived from 5 INS-1 experiments (each containing four independent samples in parallel) and from three islet experiments (each containing four independent samples in parallel).

*Preproinsulin-mRNA and preproglucagon-mRNA in INS-1 cells*

The release of relevant amounts of glucagon requires a biosynthetic backup with adequate expression of preproglucagon-mRNA (PPG-mRNA). Northern blot analysis has so far failed to detect PPG-mRNA [1], although the presence of mRNAs for all four islet hormones in INS-1 cells has been adumbrated by reverse transcription PCR techniques [9]. To specify this issue more quantitatively, the content of preproinsulin-mRNA ($cont_{PPI\text{-}mRNA}$) and of PPG-mRNA ($cont_{PPG\text{-}mRNA}$) in INS-1 cells was measured by real-time RT-PCR analysis, and the values obtained were compared to the respective mRNA contents found in primary rat pancreatic islets ($n = 18$ different INS-1 preparations).

In INS-1 cells, $cont_{PPI\text{-}mRNA}$ was $18 \pm 12\%$ of islet $cont_{PPI\text{-}mRNA}$. ($P < 0.0001$). On the other hand, $cont_{PPG\text{-}mRNA}$ was only $1.6 \pm 1.2\%$ of the respective value in islets ($P < 0.0001$; Fig. 4). Although these data demonstrate the presence of PPG-mRNA in INS-1 cells and thereby strengthen the case for an active glucagon biosynthesis in INS-1 cells, its magnitude is surely much lower than in primary islets. How can this small amount of PPG-mRNA reflect the substantial glucagon protein secretion we observed? To our knowledge, it is not known how α-cellular glucagon gene transcription, mRNA translation, and protein secretion rates are controlled. Comparable data may be drawn from insulin producing β-cells. Here, large amounts of cellular PPI-mRNA do not contribute to basal insulin biosysnthesis, but are stored in quiescent cytoplasmic pools [24]. These supplementary PPI-mRNA pools do not take part in basal insulin biosynthesis, but are only needed to amplify glucose-induced translational control in the long term [25]. In INS-1 cells, where translational control of preproinsulin biosynthesis is lacking [1], this abundance of cytoplasmic PPI-mRNA may not be required. A similar scenario can be envisioned with respect to glucagon production and PPG-mRNA levels in glucagon producing INS-1 cells.

## Conclusions

We found a substantial glucagon secretion from the polyclonal rat insulinoma cell line INS-1. While the intracellular presence of glucagon could be qualitatively

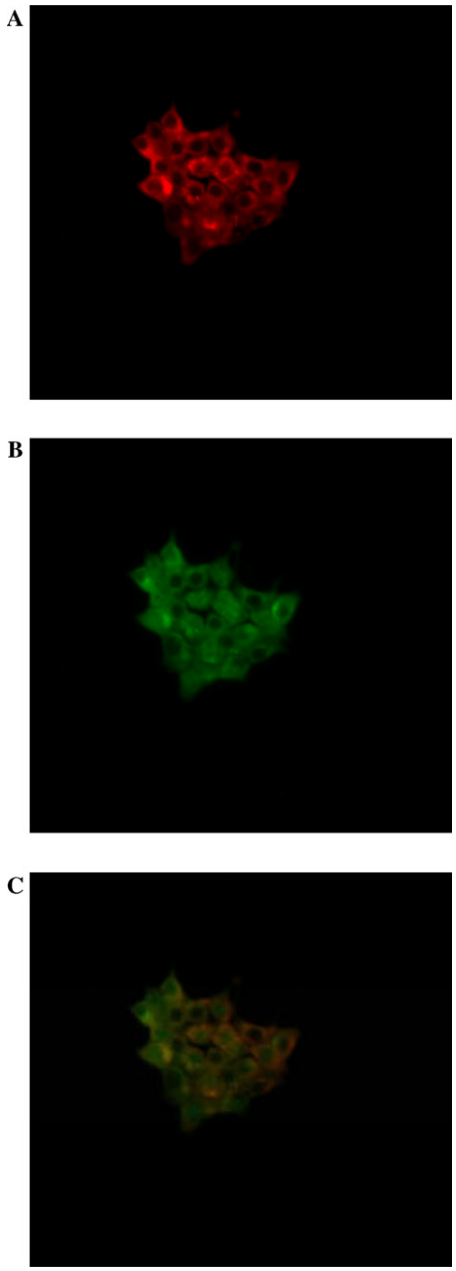


Fig. 3. Immunohistochemical analysis of plurihormonal INS-1 cells. INS-1 cells were sub-cultured on glass slides and simultaneously immuno-stained for C-peptide (via red Cy3) and glucagon (via Oregon green). A representative layer of INS-1 cells is depicted showing C-peptide (A, red), glucagon (B, green), and an overlay of both images (C, yellow).

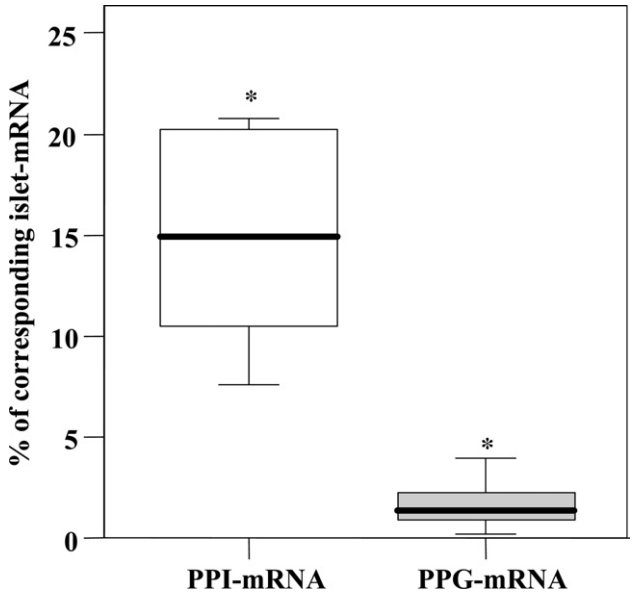


Fig. 4. Comparison of the preproglucagon-mRNA and the preproinsulin-mRNA contents in INS-1 cells and rat pancreatic islets. Preproglucagon-mRNA (PPG-mRNA) and preproinsulin-mRNA (PPI-mRNA) were quantified in 18 different total RNA preparations of INS-1 cells (within passage #51–#78) by RT-PCR and normalized by the corresponding 18S-rRNA values. The values are given relative to the expression of these genes in primary rat islets (from $n = 3$ independent experiments) as Tukey's box plots. $^{*}P \leqslant 0.05$ when compared to islet values.

confirmed by immunofluorescence experiments, the intracellular glucagon and the PPG-mRNA contents were quantitatively low in comparison to primary rat pancreatic islets. Glucose did not regulate glucagon secretion from INS-1 cells, whereas palmitate induced a threefold increase of glucagon release. From these data, one must conclude that even the low level of PPG-mRNA expression is sufficient to provide enough glucagon biosynthesis needed for a glucagon release of almost half of that seen in primary islets. This secretory activity, however, is not backed up by high intracellular glucagon stores. Taking the lacking glucose regulation of glucagon secretion into account, it can be speculated that most of the glucagon production is secreted constitutively. However, as the stimulatory action of FFA is also consistent with a nutrition-regulated secretion of glucagon in INS-1 cells, this issue needs further investigation.

## Acknowledgments

The authors acknowledge the excellent technical assistance of Joachim Schweimer. The work was supported by a grant from the Deutsche Forschungsgemeinschaft (DFG) (Bo 1379/2-2).

## References

[1] M. Asfari, D. Janjic, P. Meda, G. Li, P.A. Halban, C.B. Wollheim, Establishment of 2-mercaptoethanol-dependent differentiated insulin-secreting cell lines, Endocrinology 130 (1992) 167–178.

[2] C.B. Newgard, While tinkering with the β-cell...metabolic regulatory mechanisms and new therapeutic strategies: American Diabetes Association Lilly Lecture, 2001, Diabetes 51 (2002) 3141–3150.

[3] D. Janjic, P. Maechler, N. Sekine, C. Bartley, A.S. Annen, C.B. Wolheim, Free radical modulation of insulin release in INS-1 cells exposed to alloxan, Biochem. Pharmacol. 57 (1999) 639–648.
[4] H.E. Hohmeier, H. Mulder, G. Chen, R. Henkel-Rieger, M. Prentki, C.B. Newgard, Isolation of INS-1-derived cell lines with robust ATP-sensitive $K^+$ channel-dependent and -independent glucose-stimulated insulin secretion, Diabetes 49 (2000) 424–430.
[5] A. Merglen, S. Theander, B. Rubi, G. Chaffard, C.B. Wollheim, P. Maechler, Glucose sensitivity and metabolism-secretion coupling studied during two-year continuous culture in INS-1E insulinoma cells, Endocrinology 145 (2004) 667–678.
[6] G. Kloppel, P.U. Heitz, Pancreatic endocrine tumors, Pathol. Res. Pract. 183 (1988) 155–168.
[7] J. Roth, G. Kloppel, O.D. Madsen, M.J. Storch, P.U. Heitz, Distribution patterns of proinsulin and insulin in human insulinomas: an immunohistochemical analysis in 76 tumors, Virchows Arch. B Cell Pathol. Incl. Mol. Pathol. 63 (1992) 51–61.
[8] A.J. Williams, P.J. Coates, D.G. Lowe, C. McLean, E.A. Gale, Immunochemical investigation of insulinomas for islet amyloid polypeptide and insulin: evidence for differential synthesis and storage, Histopathology 21 (1992) 215–223.
[9] H. Wang, P. Maechler, B. Ritz-Laser, K.A. Hagenfeldt, H. Ishihara, J. Philippe, C.B. Wollheim, Pdx1 level defines pancreatic gene expression pattern and cell lineage differentiation, J. Biol. Chem. 276 (2001) 25279–25286.
[10] E. Samols, S. Bonner-Weir, G.C. Weir, Intra-islet insulin–glucagon–somatostatin relationships, Clin. Endocrinol. Metab 15 (1986) 33–58.
[11] K.M. Hope, P.O. Tran, H. Zhou, E. Oseid, E. Leroy, R.P. Robertson, Regulation of alpha-cell function by the β-cell in isolated human and rat islets deprived of glucose: the "switch-off" hypothesis, Diabetes 53 (2004) 1488–1495.
[12] L.C. Bollheimer, S. Troll, H.W.C. Landauer, J. Schölmerich, R. Buettner, Insulin-sparing effects of troglitazone in rat pancreatic islets, J. Mol. Endocrinol. 31 (2003) 61–69.
[13] J. Xiao, S. Gregersen, S.B. Pedersen, K. Hermansen, Differential impact of acute and chronic lipotoxicity on gene expression in INS-1 cells, Metabolism 51 (2002) 155–162.
[14] V. Poitout, L.K. Olson, R.P. Robertson, Insulin-secreting cell lines: classification, characteristics and potential applications, Diabetes Metab. 22 (1996) 7–14.
[15] R.H. Unger, Glucagon physiology and pathophysiology in the light of new advances, Diabetologia 28 (1985) 574–578.
[16] S. Banarer, V.P. McGregor, P.E. Cryer, Intraislet hyperinsulinemia prevents the glucagon response to hypoglycemia despite an intact autonomic response, Diabetes 51 (2002) 958–965.
[17] L.C. Bollheimer, H.C. Landauer, S. Troll, J. Schweimer, C.E. Wrede, J. Schölmerich, R. Buettner, Stimulatory effects of free fatty acids on glucagon secretion at low to normal glucose concentrations, Metabolism 53 (2004) 1443–1448.
[18] C.S. Olofsson, A. Salehi, S.O. Gopel, C. Holm, P. Rorsman, Palmitate stimulation of glucagon secretion in mouse pancreatic alpha-cells results from activation of L-type calcium channels and elevation of cytoplasmic calcium, Diabetes 53 (2004) 2836–2843.
[19] L. Segall, N. Lameloise, F. Assimacopoulos-Jeannet, E. Roche, P. Corkey, S. Thumelin, B.E. Corkey, M. Prentki, Lipid rather than glucose metabolism is implicated in altered insulin secretion caused by oleate in INS-1 cells, Am. J. Physiol 277 (1999) E521–E528.
[20] G.C. Yaney, B.E. Corkey, Fatty acid metabolism and insulin secretion in pancreatic beta cells, Diabetologia 46 (2003) 1297–1312.
[21] C.J. Greenbaum, P.J. Havel, G.J. Taborsky Jr., L.J. Klaff, Intra-islet insulin permits glucose to directly suppress pancreatic A cell function, J. Clin. Invest 88 (1991) 767–773.
[22] H. Ishihara, P. Maechler, A. Gjinovci, P.L. Herrera, C.B. Wollheim, Islet beta-cell secretion determines glucagon release from neighbouring alpha-cells, Nat. Cell Biol. 5 (2003) 330–335.
[23] A. Wendt, B. Birnir, K. Buschard, J. Gromada, A. Salehi, S. Sewing, P. Rorsman, M. Braun, Glucose inhibition of glucagon secretion from rat alpha-cells is mediated by GABA released from neighboring beta-cells, Diabetes 53 (2004) 1038–1045.
[24] K.A. Goodge, J.C. Hutton, Translational regulation of proinsulin biosynthesis and proinsulin conversion in the pancreatic beta-cell, Semin. Cell Dev. Biol. 11 (2000) 235–242.
[25] B. Wicksteed, C. Alarcon, I. Briaud, M.K. Lingohr, C.J. Rhodes, Glucose-induced translational control of proinsulin biosynthesis is proportional to preproinsulin mRNA levels in islet beta-cells but not regulated via a positive feedback of secreted insulin, J. Biol. Chem. 278 (2003) 42080–42090.