Mechanism of the beneficial and protective effects of exenatide in diabetic rats

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Abstract

Glucagon-like peptide 1 (GLP1) agonists are promising therapeutic agents in the treatment of diabetes mellitus. This study examines the mechanism of the protective effects of exenatide in experimental diabetes, employing four groups of ten rats each, in which two groups were streptozotocin-induced diabetic and two were control groups. One control and one diabetic group were treated with exenatide (1 μg/kg body weight (BW)) for 10 weeks. Blood plasma was taken for biochemical analyses while pancreatic tissue was taken for immunofluorescence and immunoelectron microscopy studies and real-time PCR to examine the expression of genes. The results show that exenatide improved BW gain and reduced blood glucose in diabetic rats compared with controls. Similarly, exenatide enhanced insulin release from the pancreatic fragments and improved liver and kidney functions and lipid profile in diabetic rats compared with controls. Exenatide not only induced significant increases in serum insulin level but also elevated the number of insulin-, GLP1- and exenatide-positive cells compared with untreated controls. Exenatide also elevated the number of catalase- and glutathione reductase-positive cells in diabetic rat pancreas compared with controls. Exenatide caused significant elevation in the expressions of pancreatic duodenal homeobox-1, heat shock protein-70, glutathione peroxidase, insulin receptor and GLP1 receptor genes in the pancreas of both control and diabetic rats compared with untreated animals. The results have demonstrated that exenatide can exert its beneficial and protective effects by elevating the levels of endogenous antioxidants and genes responsible for the survival, regeneration and proliferation of pancreatic β-cell.

Key Words

► islet cells
► diabetes
► insulin
► gene expression
► immunohistochemistry

Introduction

Diabetes mellitus (DM) is a major metabolic disorder and a global health problem currently affecting more than 250 million people worldwide (Zimmet & Alberti 2006). Type 1 and type 2 DM (T1DM and T2DM) can lead to a number of long-term complications including nephropathy, retinopathy, neuropathy, exocrine glands disorder, foot ulcers and cardiomyopathy (Zimmet & Alberti 2006). These complications are mainly related to insulin
deficiency in T1DM or insulin resistance or a combination of both in T2DM, resulting in prolonged hyperglycaemia, hyperlipidaemia and exaggerated levels of reactive oxygen species (ROS) with an impaired antioxidant defence system (D’Souza et al. 2009, Figueroa-Quevedo & Agil 2011).

Although exogenous insulin is used in T1DM, the majority of T2DM patients can be managed with changes in lifestyle habits and hypoglycaemic drugs (Haan 2006). In addition to these current treatment modalities, other novel ways must also be explored to improve insulin secretion endogenously (Gedulin et al. 2005).

The gastrointestinal (GI) tract plays an important role not only in the digestion and absorption of food but also in the secretion of a number of regulatory hormones. The GI tract is classified as both an endocrine and an exocrine system with much interaction between the two parts (Shetzline & Liddle 2002). The GI tract secretes a number of hormones including glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 (GLP1; Drucker 2003b). These endogenous hormones are called incretins because they can elevate the plasma insulin level after a meal. Incretins exert these effects by stimulating insulin release from the β-cells of the endocrine pancreas as well as potentiating glucose-induced insulin release (Holst 2008). GLP1 is a natural incretin consisting of about 30 amino acids, which is produced by intestinal L-cells in the GI tract, following a meal (Baggio & Drucker 2007). GLP1 is believed to play a major role in the synthesis and release of insulin in pancreatic β-cells (Holst 2006, Dorsam & Gutkind 2007). GLP1 can also suppress the release of glucagon from pancreatic α-cells (Holst 2006). Following its release from intestinal cells, GLP1 is quickly destroyed by dipeptidyl peptidase-IV (DPP4; Holst 2007, Lotty et al. 2011), and with a half-life of just 2 min, GLP1 is not very effective for therapeutically provoking the release of insulin from β-cells on a long-term basis (Gautier et al. 2008). Thus, newly modified GLP1 analogues have been synthesised to replace GLP1. One such synthetic incretin is exenatide, an artificial form of the natural exendin-4. Exendin-4 was initially extracted from the salivary juice of the lizard, Gila monster (Heloderma suspectum) and was shown to possess about 53% of the amino acid chain of GLP1 (Baggio & Drucker 2007). There is much evidence that exenatide can mimic the effect of GLP1, and in addition, it is resistant to DPP4, and thus has a prolonged active half-life compared with GLP1 (Barnett 2007). Previous studies have shown that exenatide can induce anti-apoptotic effect in pancreatic β-cells and that it possesses GLP1-like effects on the endocrine pancreas. Therefore, this study was designed to investigate the mechanisms by which exenatide exerts its beneficial and protective effects in the rat pancreas during streptozotocin (STZ)-induced T1DM.

Materials and methods

Animals

This study employed young male Wistar rats weighing ~250 g. The animals were obtained from the College of Medicine and Health Sciences (CMHS), United Arab Emirates University, breeding colony and the Animal Research Group’s guidelines for the care and use of laboratory animals were followed. All rats were housed in temperature- (25 °C) and humidity-controlled rooms with 12 h light:12 h darkness photoperiod cycle. The animals were supplied with a standard rat chow and tap water and allowed to eat and drink ad libitum. This study had ethical clearance from the Ethics Committees of the University of Central Lancashire (UCLAN) and CMHS to carry out the experiments.

Induction of experimental diabetes

DM was induced in young adult male rats by a single i.p. injection of (0.3 ml) STZ (Sigma) at a dose of 60 mg/kg body weight (BW) (Adeghate 1999). The STZ was freshly dissolved in citrate buffer (0.5 M, pH 4.5). Age-matched healthy control rats received the same volume (0.3 ml) of the citrate buffer only. Five days after injection of either STZ or citrate buffer, the rats were tested for DM using a drop of blood from the tail vein. The estimation of blood glucose level was made using Optium Xceed Glucometer (Abbott Laboratories, Abbott Park, IL, USA) for each rat. The rats level was made using Optium Xceed Glucometer (Abbott Laboratories, Abbott Park, IL, USA). All measurements except BW and fasting blood glucose levels were performed at the end of the experiment.

Weight measurement The weight of normal and diabetic rats was recorded every 2 weeks using a 9001 Scale (Satorius, Hertfordshire, UK).
Fasting blood glucose measurement

The fasting blood glucose level was measured every 2 weeks for each individual rat of all groups using samples collected from the tail vein after an overnight fast.

Intraperitoneal glucose tolerance test

At the end of 10 weeks of treatment, age-matched, healthy controls and STZ-induced diabetic rats were subjected to an intraperitoneal glucose tolerance test (IPGTT), after overnight fasting for 18 h. Each rat from the four groups was given an i.p. glucose load of 2 g/kg BW according to a previously published procedure (Caluwaerts et al. 2007). Blood glucose measurements were made after fasting at zero time (before glucose load), 30, 60, 120 and 180 min after the glucose load.

Tissue and blood collection and tissue processing

At the end of the experiments, all ten rats from each group were subjected to general anaesthesia by diethyl ether. Following decapitation, blood was collected in heparinised tubes. The rats were dissected and the pancreas was rapidly removed. Representative pancreatic fragments were taken and used for both morphological, in vitro study and molecular biological investigation.

Biochemical analysis

Serum levels of aspartate aminotransferase, alanine aminotransferase, creatinine, uric acid cholesterol and serum triglyceride and blood urea nitrogen were determined using the Beckman Coulter Synchron UniCel, DxC, 800 Synchron, Clinical System (Brea, CA, USA).

Serum insulin estimation by ELISA

Serum insulin level was determined by using a commercial DakoCytomation Kit (Glostrup, Denmark), an ELISA method was based on two MABs. Values were expressed as μIU/ml.

Estimation of in vitro pancreatic insulin release

The pancreas from each rat in each group was removed and placed in PBS. The in vitro pancreatic insulin release was determined according to a previously described method (Adeghate & Ponery 2002).

Immunohistochemical studies

The isolated pancreas from each rat in each group was trimmed free of connective tissues and processed for immunohistochemistry using a previously described method (Adeghate & Ponery 2004) with antibodies against insulin, glucagon, catalase, glutathione reductase (GSR), GLP1 and exenatide (Table 1). The antibodies for insulin, glucagon, GLP1, exenatide, GSR and catalase were purchased from commercial suppliers. No specific immunostaining was observed in pancreatic tissue sections when primary antibodies were omitted.

Morphometric analysis

The number of insulin-, glucagon-, GLP1-, exenatide-, glutathione reductase- and catalase-positive cells was counted and estimated semi-quantitatively in treated and untreated normal and diabetic rat pancreatic tissues.

Immunofluorescence studies

Isolated pancreatic tissues were retrieved, fixed and embedded in paraffin and subsequently processed for double-labelling immunofluorescence using a previously described method (Adeghate & Ponery 2004) with antibodies against insulin, glucagon, catalase, glutathione reductase, GLP1 and exenatide (Table 1).

Immunoelectron microscopy study

Nickel grids with sections of pancreatic islets were immersed in 0.01 M sodium citrate buffer at 90 °C for

Table 1  Antibodies employed in this study

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Type</th>
<th>Cat no.</th>
<th>Dilution</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-insulin</td>
<td>Guinea pig</td>
<td>Polyclonal</td>
<td>A0564</td>
<td>1:1000</td>
<td>DakoCytomation</td>
</tr>
<tr>
<td>Anti-glucagon</td>
<td>Rabbit</td>
<td>Polyclonal</td>
<td>A0565</td>
<td>1:1000</td>
<td>DakoCytomation</td>
</tr>
<tr>
<td>Anti-GLP1</td>
<td>Rabbit</td>
<td>Polyclonal</td>
<td>H-028-13</td>
<td>1:1000</td>
<td>Phoenix Pharmaceuticals</td>
</tr>
<tr>
<td>Anti-exenatide</td>
<td>Rabbit</td>
<td>Polyclonal</td>
<td>H-070-94</td>
<td>1:1000</td>
<td>Phoenix Pharmaceuticals</td>
</tr>
<tr>
<td>Anti-glutathione reductase</td>
<td>Mouse</td>
<td>Monoclonal</td>
<td>SAB4200182</td>
<td>1:100</td>
<td>Sigma–Aldrich</td>
</tr>
<tr>
<td>Anti-catalase</td>
<td>Rabbit</td>
<td>Polyclonal</td>
<td>SAB4503383</td>
<td>1:200</td>
<td>Sigma–Aldrich</td>
</tr>
</tbody>
</table>
5 min, cooled and jet washed with deionised water. They were later processed for immunoelectron microscopy according to an established method (Mayhew & Lucocq 2011) using antibodies against either insulin/exenatide or glucagon/exenatide.

Real-time PCR

Estimation of the levels of gene expression was done using relative quantification (RQ) assay by real-time PCR (RT-PCR) technique (Wong & Medrano 2005). RQ was used to compare expression levels of genes in pancreatic tissues of treated and untreated rats. Total RNA was extracted from rat pancreatic tissues using a Ribopure Extraction Kit (Ambion–Applied Biosystems, Grand Island, NY, USA) according to the manufacturer's recommendations. cDNA was synthesised using a high-capacity cDNA RT kit with RNAase inhibitor (Applied Biosystems). Gene expression assays using TaqMan labelled primers and probes were undertaken using relative RT-PCR using an Applied Biosystems 7500 RT-PCR System (Applied Biosystems). All primers and TaqMan probes (Table 2) were purchased from Applied Biosystems. PCRs were carried out using the following cycling conditions: as one cycle at 50°C for 2 min then one cycle at 95°C for 10 min followed by 40 cycles at 95°C for 15 s and 60°C for 1 min (Hamouchene et al. 2011). Data were analysed by creating a RQ study using the Sequence Detection Systems (SDS) software of a 7500 ABI instrument (Applied Biosystems). The RQ technique was employed to determine the change in the expression of the target gene in the treated sample relative to the untreated sample. The results were calculated as a fold difference of the gene amplification values after their normalisation to the levels of the endogenous β-actin gene expression.

Table 2  PCR primers (F, forward; R, reverse) and probes (P) used in this study

<table>
<thead>
<tr>
<th>Gene</th>
<th>Species</th>
<th>Reference sequence</th>
<th>Primer and probe sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pancreatic duodenal homeobox-1 (PDX1)</td>
<td>Rat</td>
<td>NM_022852.3</td>
<td>F: GGCTTAACCTAAGCGGCAACA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: GGGAGCGTCGCAAGGTTGGA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P: TGAAAACTTGAGGGCTGCT</td>
</tr>
<tr>
<td>Heat shock protein-70 (HSP70)</td>
<td>Rat</td>
<td>NM_153629.1</td>
<td>F: AGTTGCCAAGCCTGAAGTAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: CAAAGAATTTGACCAAGCAG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P: GACCTGCAATGAGCAGCAA</td>
</tr>
<tr>
<td>Glutathione peroxidase (GPx)</td>
<td>Rat</td>
<td>NM_183403.2</td>
<td>F: TGCCCTACCTTTATGACGAC</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: TCGATGTGATGCTGTGAGGAA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P: CATTCCTCCTATGACAGGAT</td>
</tr>
<tr>
<td>Glucagon (GCG)</td>
<td>Rat</td>
<td>NM_012707.2</td>
<td>F: ACAACACTTGCCAAAGCTCA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: CAGCTATGGCGACCTTTCC</td>
</tr>
<tr>
<td>Insulin receptor (INSr)</td>
<td>Rat</td>
<td>NM_017071.2</td>
<td>F: TGAATTGAGGGCGATGGCTG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R: AAAGTTGCCAACACATCTG</td>
</tr>
<tr>
<td>Glucagon-like peptide 1 receptor (GLP1r)</td>
<td>Rat</td>
<td>NM_012728.1</td>
<td>F: ATCCACCTGAAATGCCTTGGC</td>
</tr>
<tr>
<td>β-actin (ACTB)</td>
<td>Rat</td>
<td>NM_031144.2</td>
<td>F: CCACACTGTGCCACACTACTAG</td>
</tr>
</tbody>
</table>

Statistical analysis

All values were calculated as mean ± S.E.M. Student’s t-test was used to analyse the significance of differences between mean values and different groups were assessed using SPSS Statistical Analysis Software. Differences with P<0.05 were accepted as significant when comparing control and treated samples. Differences with either P<0.01 or P<0.001 were accepted as moderately or highly significant respectively.

Results

Metabolic parameters

Figure 1A, B and C shows the time courses of changes in body weight (BW), blood glucose level and IPGTT in exenatide-treated (10 µg/kg BW) and untreated diabetic and age-matched control rats over the 10 weeks of the study. The results show that untreated diabetic rats gained...
Effect of exenatide on insulin release

Figure 2A and B shows the effect of treating pancreatic fragments with different concentrations (10^{-12}, 10^{-9} and 10^{-6} M) of exenatide on insulin release using samples from age-matched control and diabetic animals after incubation for 1 h with each concentration of the secretagogue. The results show that exenatide can stimulate insulin release from pancreatic fragments in a dose-dependent manner with significantly (P<0.001) higher release from either control or diabetic fragments compared with basal levels. In addition, insulin release from exenatide-treated control fragments was significantly (P<0.001) higher than that from exenatide-treated diabetic rat pancreas. Similarly, pancreatic fragments of untreated control rats secreted significantly (P<0.001) more insulin compared with those from untreated diabetic animals.

Effect of exenatide on serum insulin level and liver and kidney function

Table 3 shows the levels of serum insulin, aspartate aminotransferase, alanine aminotransferase, creatinine, uric acid, cholesterol and triglycerides and blood urea nitrogen in normal untreated, normal exenatide-treated, diabetic untreated and diabetic exenatide-treated rats at the end of 10 weeks following treatment. The results show that exenatide treatment can result in a significant (P<0.05) increase in serum insulin compared with untreated control or untreated diabetic rats. In addition, exenatide treatment led to significant (P<0.05, P<0.01 or
**Table 3** Serum levels of insulin, aspartate and alanine aminotransferases, blood urea nitrogen, serum creatinine, uric acid and serum cholesterol in normal untreated, exenatide-treated normal, untreated diabetic and exenatide-treated diabetic rats

<table>
<thead>
<tr>
<th>Biochemical parameters</th>
<th>Normal untreated</th>
<th>Normal exenatide-treated</th>
<th>Diabetic untreated</th>
<th>Diabetic exenatide-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum insulin (µU/ml)</td>
<td>5.9 ± 0.3</td>
<td>6.9 ± 0.3†</td>
<td>3.5 ± 0.2</td>
<td>4.7 ± 0.1‡</td>
</tr>
<tr>
<td>Serum aspartate aminotransferase (UI/l)</td>
<td>217 ± 10.1</td>
<td>85 ± 7.6†</td>
<td>444 ± 23.8</td>
<td>211 ± 28.2†</td>
</tr>
<tr>
<td>Serum alanine aminotransferase (UI/l)</td>
<td>33 ± 2.5</td>
<td>22 ± 2.1§</td>
<td>192 ± 22.4</td>
<td>91 ± 12.7‡</td>
</tr>
<tr>
<td>Blood urea nitrogen (mg/dl)</td>
<td>15.8 ± 1.1</td>
<td>14.9 ± 1.1</td>
<td>28.7 ± 1.0</td>
<td>25.8 ± 1.5</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl)</td>
<td>0.45 ± 0.03</td>
<td>0.35 ± 0.04†</td>
<td>0.56 ± 0.04</td>
<td>0.45 ± 0.02*</td>
</tr>
<tr>
<td>Serum uric acid (mg/dl)</td>
<td>1.4 ± 0.1</td>
<td>0.92 ± 0.12†</td>
<td>2.4 ± 0.36</td>
<td>0.8 ± 0.15§</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>29 ± 1.1</td>
<td>23 ± 2.4†</td>
<td>49 ± 2.4</td>
<td>44 ± 5.0</td>
</tr>
<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>47 ± 3.6</td>
<td>24 ± 2.1§</td>
<td>79 ± 6.7</td>
<td>48 ± 5.9§</td>
</tr>
</tbody>
</table>

* or † P<0.05 vs untreated animals, ‡ or § P<0.01 vs untreated animals and † or ‡ P<0.001 vs untreated animals.

**Effect of exenatide in diabetic rats**

Exenatide treatment resulted in significant (P<0.05) decreases in serum aspartate aminotransferase, serum alanine aminotransferase, serum creatinine, serum uric acid and serum triglyceride in both control and diabetic rats compared with the respective untreated control and untreated diabetic rats. The results also show that exenatide treatment can cause a small but not significant decrease in serum cholesterol in treated diabetic animals compared with untreated diabetic animals. However, in control animals, exenatide can significantly (P<0.05) reduce serum cholesterol levels compared with those of untreated control rats. In contrast, exenatide has no effect on blood urea nitrogen in either control or diabetic animals compared with the respective untreated control rats.

**Effect of exenatide on insulin-, catalase- and glutathione reductase-containing cells**

Figure 3 shows that exenatide treatment had no significant effect on either catalase, glutathione reductase or exenatide-positive cells in the pancreas of exenatide-treated control rats compared with untreated control. However, exenatide treatment resulted in significant (P<0.05) increases in catalase, glutathione reductase and exenatide immunopositive cells in the diabetic rat pancreas compared with those of untreated diabetic animals.

**Immunofluorescence double labelling**

Figure 4 shows micrographs of immunofluorescence double labelling of the co-localisation of insulin with exenatide, GLP1, catalase or glutathione reductase in the pancreatic islets of normal and diabetic rats. The results show that insulin is co-localized with exenatide, GLP1, catalase and glutathione reductase in the islet of Langerhans.

**Effects of exenatide on the number of insulin-, glucagon-, GLP1-, exenatide-, catalase- and GSR-immunopositive cells in the pancreas of untreated normal, exenatide-treated normal, untreated diabetic and exenatide-treated diabetic rats**

Figure 5A, B, C, D, E and F shows the effects of exenatide treatment on the percentage distribution of insulin-immunopositive cells, glucagon-immunopositive cells, GLP1-immunoreactive cells, exenatide-immunoreactive cells, catalase-immunopositive cells and glutathione reductase-immunopositive cells in the pancreas of untreated normal, exenatide-treated normal, untreated diabetic and exenatide-treated diabetic rats, respectively. The results show that exenatide treatment can result in significant (P<0.05) increases in the number of insulin and GLP1-immunopositive cells in the pancreas of both exenatide-treated control and diabetic rats compared with untreated controls. Untreated diabetic rats had significantly (P<0.05) lower numbers of insulin-positive cells and significantly (P<0.05) more GLP1-positive cells compared with control rats. In contrast, exenatide treatment resulted in significant (P<0.05) decreases in the percentage distribution of glucagon-positive cells in both control and diabetic animals. Moreover, the diabetic rats’ pancreases had significantly (P<0.001) more glucagon-positive cells compared with those of control animals.
Immunoelectron microscopy

Immunoelectron microscopy was carried out to visualise the intracellular localization of exenatide and other molecules with which it co-localized. Figure 6 shows immunogold-labelled exenatide in association with insulin- and glucagon-labelled particles in the secretory granules of the pancreatic β- and α-cells, respectively. The results show that exenatide co-localizes with both insulin and glucagon in the endocrine pancreas.

Effect of exenatide on gene expression of selected molecules

Figure 7 shows the effects of exenatide treatment on gene expression of (A) pancreatic duodenal homeobox-1, (B) heat shock protein-70 (HSP70), (C) glutathione peroxidase (GPx), (D) glucagon, (E) insulin receptor and (F) GLP1 receptor (GLP1r) in the pancreas of age-matched untreated-normal, exenatide-treated normal, diabetic untreated and exenatide-treated diabetic rats, respectively. The results...
Figure 4
Micrographs of immunofluorescence double labelling showing the distribution of (I) insulin-positive cells (green; A, D, G, J) and exenatide-positive cells (red; B, E, H, K), (II) insulin-positive cells (green; A, D, G, J) and GLP1-positive cells (red; B, E, H, K), (III) insulin-positive cells (green; A, D, G, J) and catalase-positive cells (red; B, E, H, K) and (IV) insulin-positive cells (green; A, D, G, J) and glutathione reductase-positive cells (red; B, E, H, K) in the pancreatic islet of normal and diabetic rats, n = 10 for each. (C, F, I, L) merged images for groups I–IV. Magnification, ×400.
reveal that exenatide treatment can induce significant ($P<0.05$) increases in the gene expression of pancreatic duodenal homeobox-1, HSP70, GPx, insulin receptor and GLP1r in both control and diabetic rats. Moreover, the results further show that exenatide treatment can significantly ($P<0.01$) reduce glucagon gene expression in both control and diabetic rats compared with untreated animals.

**Discussion**

Research into methods that can protect pancreatic $\beta$-cells from damage and help them to produce more endogenous insulin is of paramount importance for the management of DM.

In a normal person, the pancreatic $\beta$-cells mass can adapt itself to different insulin requirements when challenged with various loads of blood glucose. Unfortunately, this process is severely compromised in DM. In T1DM, the $\beta$-cells mass and the cells themselves are either partially or fully damaged leading to a reduced level of insulin secretion. In the case of T2DM, there is a defect in the insulin molecule or the insulin receptor or reduced $\beta$-cell mass (Zimmet & Alberti 2006). All of these features suggest that the endocrine pancreas is unable to maintain adequate $\beta$-cell mass in DM. This demise of the endocrine pancreas is probably due to a number of environmental insults including life style habits, drugs and genetic factors (Adeghate et al. 2006).

The processes by which the protection, preservation and regeneration of $\beta$-cells can be maintained are of therapeutic importance for the treatment of DM. Recently, a new class of bioactive agents called incretins has been developed. These incretins can increase $\beta$-cells’ mass and survival, thus, enhancing their lifespan and optimally regulating the secretion of insulin. Naturally occurring incretin-like substances are located and secreted in the GI tract. These endogenous incretins can stimulate both the synthesis and release of insulin from pancreatic $\beta$-cells (Cernea & Raz 2011). One such substance is the naturally occurring hormone, GLP1. Unfortunately, GLP1 has a very short half-life leading to its early degradation (Lotfy et al. 2011). However, its synthetic analogue, exenatide has

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**Figure 5**

Charts showing the percentage distribution of (A) insulin-, (B) glucagon-, (C) GLP1-, (D) exenatide-, (E) catalase- and (F) glutathione reductase (GSR)-positive cells in the pancreas of normal untreated, normal exenatide-treated, untreated diabetic and exenatide-treated diabetic rats. Data are mean ± S.E.M., $n=10$, *$P<0.05$, ** or ***$P<0.01$ and ****$P<0.001$ for treated compared with untreated rats.
been shown to have a greater potential than natural GLP1 in inducing the pancreas to synthesise and produce insulin (Dungan & Buse 2005). Exenatide is the synthetic form of exendin-4, a naturally occurring peptide consisting of 39 amino acids and which was isolated from the salivary fluid of the lizard, *H. suspectum* or Gila monster (Baggio & Drucker 2007). Generally, exenatide shows a similar structure to that of human GLP1, with a similarity of 53% for the amino acid sequence (Kim & Egan 2008). Exenatide is described as an incretin mimetic polypeptide, which can activate GLP1r in pancreatic β-cell membrane (Holst 2007). The GLP1r is a G-protein-coupled receptor spanning the β-cell plasma membrane (Brubaker & Drucker 2002). One major difference between GLP1 and exenatide is the type of amino acid, which is found in position two of the molecule. GLP1 has alanine whereas exenatide has glycine. The presence of glycine makes exenatide unrecognisable by the enzyme DPP4, which is responsible for its slow degradation, thus prolonging its plasma half-life (Lotfy et al. 2011).

**Effect of exenatide on metabolic parameters**

The results of this study have demonstrated that administration of exenatide at a physiological dose to STZ-induced diabetic rats can produce both protective and beneficial effects on the animals compared with untreated diabetic rats. Similarly, exenatide exerts the same protective and beneficial effects in treated control rats compared with untreated animals. Following treatment with exenatide, the diabetic rats gained significantly more weight and moreover, they had significantly less blood glucose compared with untreated diabetic animals. Exenatide reduces weight gain in obese patients, which is beneficial to the overall management of DM patients with obesity (Vilsbøll et al. 2012). In this study, we observed that diabetic rats treated with exenatide gained weight throughout the experimental period. The weight gain may be due to the fact that exenatide improves β-cell mass and insulin release. Exenatide treatment resulted in marked and significant elevation of insulin release from both control and diabetic rat pancreas compared with the results for untreated animals. It is worth noting that exenatide was more effective in inducing insulin release from the control rat pancreas compared with that from the diabetic rat pancreas. These studies also show that exenatide acts as a powerful secretagogue to induce insulin release from the pancreas. In addition, the present results have clearly demonstrated that exenatide treatment can lead to an upregulation of gene expressions for GLP1 and

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**Figure 6**

Electron micrographs showing immunogold labelling of pancreatic cells. Images showing β-cells in (A) normal and (B) diabetic rats. Large arrows show insulin granules (20 nm gold particles) and small arrows show exenatide (15 nm gold particles). Images showing α-cells of pancreatic islets in (C) normal and (D) diabetic rats. Large arrows show glucagon granules (20 nm gold particles) and small arrows show exenatide (15 nm gold particles). The results show that exenatide is co-localised with both insulin and glucagon in the endocrine pancreas, where they lie side by side in secretory granules, *n* = 10 for each. Magnification, × 27 500.
insulin receptors, as well as pancreatic duodenal homeobox-1 in both the treated control and treated diabetic rats compared with the respective untreated controls. Similarly, exenatide treatment can result in the elevation of the number of exenatide-, insulin- and GLP1-immunopositive cells in both control and diabetic rat pancreas compared with the levels in untreated rats.

The question which now arises is how does exenatide exert its beneficial effect on the endocrine pancreas? Exenatide is believed to exert its beneficial effect in the endocrine pancreas by maintaining optimal β-cell mass and function by increasing the β-cell gene expression. In turn, this may lead to β-cell proliferation and neogenesis, thereby inhibiting apoptosis in pancreatic islet cells (Xu et al. 2009). Exenatide can also exert its secretagogue effect on insulin release by activating GLP1r in the β-cells. In addition, it was previously demonstrated that exenatide can elicit a short-term effect on pancreatic β-cell responsiveness to glucose, resulting in the release of insulin when the body is subjected to hyperglycaemia. In turn, this will subsequently lead to a concomitant reduction in both blood glucose and insulin release (Barnett 2007).

**Exenatide and liver and kidney functions**

In addition to the release of insulin from β-cells, the present results have also indicated that exenatide treatment can protect the diabetic rats from both kidney dysfunction and liver damage compared with untreated diabetic rats. Our result corroborates those of

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**Figure 7**

Charts showing the effect of exenatide treatment on the gene expressions, expressed as relative quantifications, of (A) pancreatic duodenal homeobox-1, (B) heat shock protein-70, (C) glutathione peroxidase, (D) glucagon, (E) insulin receptors and (F) glucagon-like peptide-1 receptor (GLP1r) in the pancreas of age-matched normal untreated, normal exenatide-treated, untreated diabetic and exenatide-treated diabetic rats. Data are mean ± s.e.m., n = 10, * or #P < 0.05, **P < 0.01 and *** or ###P < 0.001 for treated compared with untreated animals.
Vaghasiya et al. (2010). In exenatide-treated diabetic rats, there were significant decreases in serum levels of alanine and aspartic aminotransferases, cholesterol, triglyceride, uric acids, blood urea nitrogen and creatinine compared with untreated diabetic rats.

**Effect of exenatide on endogenous antioxidants**

Treatment of diabetic rats with exenatide can result in significant increases in the number of catalase- and glutathione reductase-positive cells and the gene expressions of GPx and HSP70 compared with untreated diabetic animals. These results clearly show that exenatide can exert a protective effect on rats, especially in the pancreas by enhancing the levels of markers and mediators that prevent the generation of ROS. The antioxidative defence mechanisms in the pancreatic β-cells are potentially weak in DM and in turn, these can be overwhelmed by redox imbalance arising from overproduction of ROS and reactive nitrogen species (Lenzen 2008). This imbalance is exacerbated further during DM. Consequences of this redox imbalance are lipid peroxidation, increased oxidative-specific proteins, DNA damage and interference of ROS with signalling pathways, all of which contribute to β-cells damage, dysfunction and subsequent death, especially during DM (Deavall et al. 2012). ROS, superoxide radicals and hydrogen peroxide are all produced during pro-inflammatory β-cell attack and glycolipid-toxicity-mediated β-cell dysfunction (Li et al. 2008). In combination with nitric oxide accumulation, these processes lead to the generation of more ROS resulting in pancreatic β-cell death (Amin et al. 2011).

**Effect of exenatide on gene expression of selected molecules**

Exenatide treatment provides protection for pancreatic β-cells during DM via elevations in the synthesis and gene expression of catalase and GPx, naturally occurring antioxidant proteins, which protect pancreatic β-cells from oxidative stress (Teixeira-Lemos et al. 2011). The beneficial effect of GPx not only targets oxidative stress but also reduces glucotoxicity by enhancing glucose tolerance. This process preserves β-cell morphology and function (Robertson & Harmon 2007).

Similarly, exenatide induced the elevation of HSP70 in diabetic rat pancreas. This protein plays a major role in reducing cell death and prolonging cell survival by stabilising denatured proteins, thereby eliminating damaged proteins for degradation or by interfering with the apoptotic process in β-cells (Muranyi et al. 2005). It is well-known that HSP70 levels are suppressed in DM, especially during insulin deficiency. As exenatide can induce insulin synthesis and release, this insulin in turn may directly elicit the expression of HSP70 leading to the protection of β-cells (Chen et al. 2006).

The results of this study also showed significant reduction in the expression of the glucagon gene in the pancreas of normal and diabetic rats treated with pharmacological doses of exenatide. Exenatide may inhibit glucagon expression and release from α-cells via GLP1r found on the plasma membrane of α-cells (Heller et al. 1997). This may in turn result in increased GLP1 synthesis from the α-cells as observed in the mouse pancreas (Kedees et al. 2009). The increased release of GLP1 can in turn stimulate insulin secretion from pancreatic β-cells (Nie et al. 2000).

A significant hike in the expression of the insulin receptor gene was also observed in the pancreases of normal and diabetic rats treated with exenatide. Signal transduction via insulin receptors on β-cells regulates the production, secretion and response of insulin to food (Fisher & White 2004, Nandi et al. 2004). Therefore, an increase in insulin receptor expression in the pancreatic β-cells is of paramount importance in the treatment of DM. The results of this study corroborate those of Park et al. (2006), who showed that exenatide uses insulin receptors to promote β-cell survival.

PDX1 is known to play an important role in the development and maturation of the pancreas and in the maintenance of normal β-cell function (Kaneto et al. 2007). In an earlier study, a significant regulatory function of the PDX1 transcription factor on the effect of GLP1r in the mouse pancreatic β-cell was presented. This report shows that exenatide can significantly enhance growth, insulin mRNA expression and insulin release in pancreatic β-cells in addition to its ability to inhibit apoptosis. In spite of these advantages, exenatide was unable to increase insulin secretion in pancreatic β-cells of Pdx1 knockout mice (Li et al. 2005). Another similar study has shown that stimulation of GLP1r signalling improves insulin gene expression (Fontes et al. 2010). Exenatide may be able to enhance β-cell transcription factor PDX1 through depression of glucolipotoxicity (McCarty 2007). The effect of exenatide on pancreatic β-cell differentiation after PDX1 stimulation may occur via protein kinase B and cAMP-dependent mechanisms (Drucker 2003a, Holz 2004). Moreover, exenatide may initiate its action on pancreatic β-cells via the stimulation of PI3K/PKB, a cell
Co-localisation of exenatide with insulin

As this study shows that exenatide is co-localised with insulin in the secretory granules of the endocrine pancreas, it may also regulate insulin metabolism at the cellular level, a molecular explanation for the protective and beneficial effect of incretins in DM. Co-localisation of GLP1 with neuropeptide PYY was recently reported in L-cells (Habib et al. 2013).

In conclusion, the results of this study have clearly demonstrated that the GLP1 analogue, exenatide can exert marked protective and beneficial effects on the endocrine pancreas of the diabetic rats, culminating in an optimal and efficient synthesis and release of insulin. This is done via a myriad of cellular and molecular pathways.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

M L, J S and E A planned and wrote the manuscript. H R contributed to the experimental data. S T contributed to the electron microscopy; E Z contributed to the gene expression studies.

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Fontes G, Hagman DK, Latour MG & Semache M 2010 Lack of preservation of insulin gene expression by a glucagon-like-peptide 1 agonist or a protecting system, and/or through the suppression of the caspase-3 apoptotic pathway (Vaghasiya et al. 2010).

Signal transduction via GLP1R plays an important role in the regulation of pancreatic β-cell differentiation and extending its lifespan. It is possible that exenatide exerts its effect via GLP1R-induced pathways. In fact, it has been shown that pancreatic islet cells failed to proliferate when GLP1R−/− mice were treated with exenatide (Deleon et al. 2003). Moreover, propagation of cell signalling and proliferation was observed when GLP1 bound to receptors on the plasma membrane of pancreatic progenitor cells (Suen et al. 2008). GLP1 is believed to act via cAMP and a PI3K-dependent system leading to an increase in β-cell mass (Salehi et al. 2008).

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