Influence of Neuropeptide Y and pancreatic polypeptide on islet function and beta-cell survival

Dawood Khan, Srividya Vasu, R. Charlotte Moffett, Nigel Irwin, Peter R. Flatt

PII: S0304-4165(17)30005-3
DOI: doi:10.1016/j.bbagen.2017.01.005
Reference: BBAGEN 28731

To appear in: *BBA - General Subjects*

Received date: 8 November 2016
Revised date: 3 January 2017
Accepted date: 4 January 2017

Please cite this article as: Dawood Khan, Srividya Vasu, R. Charlotte Moffett, Nigel Irwin, Peter R. Flatt, Influence of Neuropeptide Y and pancreatic polypeptide on islet function and beta-cell survival, *BBA - General Subjects* (2017), doi:10.1016/j.bbagen.2017.01.005

Influence of Neuropeptide Y and pancreatic polypeptide on islet function and beta-cell survival

Dawood Khan, Srividya Vasu, R. Charlotte Moffett, Nigel Irwin, Peter R. Flatt

From the SAAD Centre for Pharmacy and Diabetes, Ulster University, Coleraine, Northern Ireland, UK.

To whom correspondence should be addressed: Dr. Nigel Irwin, SAAD Centre for Pharmacy and Diabetes, School of Biomedical Sciences, University of Ulster, Coleraine, Northern Ireland, UK. E-mail: n.irwin@ulster.ac.uk. Tel: ++44 (0) 28 70 124754; Fax: ++44 (0) 28 70 123939.

Short title: NPY, PP and beta-cells

Keywords: Beta-cell, islets, Neuropeptide Y, pancreatic polypeptide, NPYR, insulin secretion, proliferation, apoptosis

Abstract

Background: In the present study we assessed the impact of neuropeptide Y receptor (NPYR) modulators, neuropeptide Y (NPY) and pancreatic polypeptide (PP), on islet function and beta-cell survival.

Methods: The effects of NPY and PP on beta-cell function were examined in BRIN BD11 and 1.1B4 beta-cells, as well as isolated mouse islets. Involvement of both peptides in pancreatic islet adaptations to streptozotocin and hydrocortisone, as well as effects on beta-cell proliferation and apoptosis was also evaluated.

Results: Neither NPY nor PP affected *in vivo* glucose disposal or insulin secretion in mice. However, both peptides inhibited ($p<0.05$ to $p<0.001$) glucose stimulated insulin secretion from rat and human beta-cells. NPY exerted similar insulinostatic effects in isolated mouse islets. NPY and PP inhibited alanine-induced changes. in BRIN BD11 cell membrane potential and $(Ca^{2+})_{i.}$ Streptozotocin treatment decreased and hydrocortisone treatment increased beta-cell mass in mice. In addition, streptozotocin, but not hydrocortisone, increased PP cell area. Streptozotocin also shifted the normal co-localisation of NPY with PP, towards more pronounced co-expression with somatostatin in delta-cells. Both streptozotocin and hydrocortisone increased pancreatic exocrine expression of NPY. More detailed *in vitro* investigations revealed that NPY, but not PP, augmented ($p<0.01$) BRIN BD11 beta-cell proliferation. In addition, both peptides exerted protective effects against streptozotocin-induced DNA damage in beta-cells.

Conclusion: These data emphasise the involvement of PP, and particularly NPY, in the regulation of beta-cell mass and function.

General significance: Modulation of PP and NPY signalling is suitable for further evaluation and possible clinical development for the treatment of diabetes.

1. Introduction

The Neuropeptide Y (NPY) family of peptides includes three biologically active hormones each comprising 36 amino acid residues, namely neuropeptide Y (NPY), pancreatic polypeptide (PP) and peptide YY (PYY) [1]. This NPY system has long been recognised to have a fundamental role in the central regulation of energy balance [2]. Indeed, the primary research focus of NPY and PP to date has been linked to effects on energy homeostasis and possible anti-obesity actions, stemming from knowledge that NPY represents a powerful orexigenic hormone, whereas PP possesses effective anorexigenic actions [3]. More recently, metabolically important peripheral actions of NPY peptides have also become apparent [2]. As such, PYY has received much recent positive attention as a potential modulator of beta-cell mass and insulin secretory function [4-7]. Receptors that mediate the biological actions of PYY, namely NPY receptors (NPYR's), are also activated by NPY and PP [8]. Specifically, NPY shows strong affinity for the NPYR1, 2 and 5, while PP is a preferential agonist at NPYR4 [9], thus together targeting all major subtypes of the NPYR family. It follows that both NPY and PP could directly influence beta-cell function and survival, in a similar manner to PYY [4].

We and others have clearly shown that NPYR's mRNA is expressed in cultured beta-cells and primary pancreatic endocrine cells, as well as direct beta-cell effects of NPYR modulators [4,7,10]. It is also well established that NPY is present in nerves that innervate the islets of Langerhans [11-13], and that NPY expression is upregulated in diabetes [14]. More recent investigations have revealed expression of NPY in various pancreatic endocrine cells, including beta-cells [15,16]. In harmony, PP is synthesised and secreted from F cells

(commonly called PP cells) of the endocrine pancreas [17], where it has been suggested to mediate satiety and act as a marker of parasympathetic activity [14]. However, the physiological relevance of the PP response to parasympathetic activation is not known. Interestingly, NPY was shown to inhibit glucose-stimulated insulin secretion (GSIS) in human and rodent islets [18]. This observation is in agreement with the recently uncovered actions of other known NPYR modulators, such as PYY(1-36) and PYY(3-36) [4]. Moreover, in accord with PYY [4,7], NPY has been suggested to have some involvement in cell proliferation and replication [19]. However, the potential mechanisms of NPY, and indeed other NPYR modulators such as PP, on beta-cell survival and function need to be fully elucidated.

Therefore, in the present study we have evaluated the effects and potential mechanisms of NPY and PP on the modulation of insulin section *in vivo* and *in vitro* from pancreatic clonal rat BRIN BD11 and cultured human 1.1B4 beta-cells, as well as isolated mouse islets. To determine the role of NPY and PP in situations of beta-cell loss and growth, relative changes in islet cell NPY and PP expression, and co-localisation with PP and δ-cells, were assessed in mice following streptozotocin and hydrocortisone treatment, that induce distinct forms of insulin-deficient and insulin-resistant diabetes. Finally, the ability of NPY and PP to positively affect beta-cell proliferation and prevent apoptosis was examined in cultured rodent and human beta-cell lines. The results support the idea that locally produced NPY and PP may have a role in the regulation of insulin secretion and preservation of beta-cell mass.

2. Materials and methods

2.1 Real time Reverse Transcription PCR

To determine pancreatic expression of NPY and PP, mRNA was extracted from isolated C57BL/6 mouse islets using an RNeasy Mini Kit following manufacturer's instructions (Qiagen, UK), as described previously [4]. Briefly, mRNA (3 µg) was converted to cDNA using SuperScript II Reverse Transcriptase kit (Invitrogen, Paisley, UK). Amplification conditions were set at 95°C for initial and final denaturation, 58°C for primer annealing and 72°C for extension for 40 cycles, followed by a melting curve analysis, with temperature range set at 60°C to 90°C. Data were analysed using ΔΔCt method and normalised to *Actb/ACTB* expression.

2.2 In vitro studies

Evaluation of the actions of NPY and PP on beta-cells were assessed *in vitro* using isolated mouse pancreatic islets and both rat and human clonal beta-cell lines. Briefly, islets were isolated from mice C57BL/6 mice by collagenase digestion, as described previously [20]. Functional studies were performed using rat BRIN BD11 and human 1.1B4 beta-cells. General culture conditions and characteristics of these cell lines have been reported previously [21-23].

2.3 In vitro insulin secretion and mechanism of action

BRIN BD11 and 1.1B4 cells were seeded (150,000/well) into 24-well plates (Nunc, Roskilde, Denmark) and allowed to attach overnight at 37°C. Following 40 min pre-incubation (1.1 mmol/L glucose; 37^{o}C), cells were incubated (20 min; 37°C) in the presence of 5.6 or 16.7 mmol/L glucose, as appropriate, with test peptides. After 20 min incubation, buffer was removed from each well and aliquots stored at -20°C prior to determination of insulin by radioimmunoassay [24]. To determine potential NPY receptor involvement, BRIN BD11 cells were incubated at 16.7 mM glucose with NPY or PP (10^{-8} and 10^{-6} M) and either the specific NPY1 receptor antagonist BVD-10 (10^{-6} M; Tocris Bioscience), the specific NPY2 receptor

antagonist BIIE 0246 (10^{-6} M; Tocris Bioscience) or the specific NPY5 receptor antagonist CGP71683 (10^{-6} M; Tocris Bioscience), and insulin secretion determined as described above. In addition, to determine potential mechanisms of action, membrane potential and intracellular Ca^{2+} were also determined following treatment with test peptides (10^{-6} M) in BRIN BD11 cells at 5.6 and 16.7 mM glucose in the absence and presence of alanine (10 mM) as described previously [25]. Briefly, BRIN BD11 cells were incubated at 37 °C, for 2 h with either FLIPR membrane potential assay kit or FLIPR Ca^{2+} assay kit (Molecular Devices, Sunnyvale, USA), to a final volume of 200 µl. Fluorimetric data were acquired using a FlexStation scanning fluorimeter with integrated fluid transfer workstation (Molecular Devices, Sunnyvale, CA). Excitation, emission, and cut-off filters were set to 530, 565 and 550 nm for membrane potential, and 485, 525 and 515 nm for Ca^{2+}. The FlexStation was set to run for 5 min, collecting data at 2.5-s interval, with test solutions transferred at a rate of 78 µl/s. Furthermore, insulin secretion from pancreatic islets in response to NPY and PP (10^{-10} – 10^{-6} M) at 16.7 mM glucose was determined as above, but with a 60 min test incubation period. Following removal of the test solution, 200 µl of acid–ethanol solution (1.5% (v/v) HCl, 75% (v/v) ethanol, 23.5% (v/v) H_2O) was added to islets for overnight extraction of cellular insulin. All samples were stored at -20°C for measurement of insulin concentrations by radioimmunoassay [24].

2.4 In vitro beta-cell proliferation and cellular stress studies

To assess the effects of NPY and PP on rodent BRIN-BD11 and human 1.1B4 beta-cell proliferation, cells were seeded at a density of 150,000 cells per well and cultured overnight in the presence of NPY or PP (10^{-6} M), and compared to positive control GLP-1 (10^{-6} M). Cells were washed with PBS and fixed using 4 % paraformaldehyde. After antigen retrieval with citrate buffer at 95°C for 20 min, tissue was blocked using 2% BSA for 45 minutes. The slides were then incubated with rabbit anti-Ki-67 primary antibody, and subsequently with Alexa

Fluor® 594 secondary antibody. Slides were viewed using fluorescent microscope (Olympus System Microscope, model BX51; Southend-on-Sea, UK) and photographed by DP70 camera adapter system. Proliferation frequency was determined in a blinded fashion and expressed as % of total cells analysed. Approximately 150 cells per replicate were analysed. For analysis of ability of NPY and PP to protect against streptozotocin-induced DNA damage, BRIN-BD11 and 1.1B4 cells were seeded as above. Cells were then exposed to streptozotocin (5 mM) in the presence or absence of NPY or PP (10^{-6} M) for 2 hours, with GLP-1 (10^{-6} M) as positive control. Cells were then harvested and a comet assay was performed as described previously [26]. Resulting gels were stained using DAPI (4', 6 – diamidino – 2 –phenylindole) (100 µg/ml) and slides were viewed under appropriate filter using an Olympus fluorescent microscope. Comet score software (Version 1.5) was used for the analysis of % tail DNA (100 cells per gel) and olive tail moment.

2.5 Animals

All animal studies were carried out using adult male C57BL/6 mice (12-14 weeks of age, Harlan Ltd, UK), housed individually in air conditioned room at 22±2°C with 12 hours light and dark cycle and *ad libitum* access to standard rodent diet (10% fat, 30% protein and 60% carbohydrate; Trouw Nutrition, Northwich, UK) and drinking water. All experiments were carried out in accordance with the UK Animal Scientific Procedures Act 1986.

2.6 Acute in vivo glucose homeostatic, insulin secretory and appetite suppressive effects

Plasma glucose and insulin responses were evaluated after intraperitoneal (i.p.) injection of glucose alone (18 mmol/kg body weight) or in combination with test peptides (25 nmol/kg body weight) in overnight (18 h) fasted C57BL/6 mice. In a second series of experiments, 18 h fasted mice were used to assess the effects of respective test peptides on food intake. Mice

received an i.p. injection of saline alone (0.9 % (w/v) NaCl) or in combination with test peptides (25 nmol/kg body weight) and food intake measured at 30 min intervals for 180 min. A dose of 25 nmol/kg was chosen based on our previous positive experience with other NPYR modulators on glucose homeostasis, insulin secretion and feeding at this dose [4].

2.7 Islet histology in insulin-deficient and insulin-resistant diabetic mice

To induce insulin-deficient diabetes, multiple low dose streptozotocin (50 mg/kg body weight, i.p.) in 0.1 M sodium citrate buffer (pH 4.5) or saline vehicle (0.9% w/v NaCl, i.p.) was injected daily (13:00 h, n=6, fasted for 4 h) for 5 days in C57BL/6 mice. Pancreatic tissues were excised 5 days after the final injection. To induce insulin-resistant diabetes, hydrocortisone was injected i.p. (70 mg/kg body weight in saline, n=6) once daily for 10 days in C57BL/6 mice, and pancreatic tissues extracted at the end of the treatment period. All pancreatic tissues were immediately fixed in 4% PFA for 48 h at 4°C and subsequently dehydrated using a series of increasing strength ethanol solutions and processed for embedding in paraffin wax using an automated tissue processor **(**Leica TP1020, Leica Microsystems, Nussloch, Germany), as described previously (Moffett *et al*. 2013; Vasu *et al*. 2014). Tissue blocks were sectioned (8 μm) using a Shandon Finesse 325 microtome (Thermo Scientific, Hemel Hempstead, UK) and picked for staining at intervals of 10 sections. After deparaffinising, sections were rehydrated using a series of decreasing strength ethanol solutions. Antigen retrieval was carried out using a citrate buffer (pH 6.0) at 94°C for 20 min, sections were then blocked using 2% BSA and incubated overnight at 4°C with appropriate primary antibody (Table 1). The slides were then incubated with appropriate secondary antibodies (Alexa Fluor® 594 for red and Alexa Fluor® 488 for green; Table 1) and stained with nuclear DAPI staining. Slides were mounted with anti-fade mounting medium and viewed using a fluorescent microscope (Olympus System Microscope, model BX51; Southend-on-Sea,

UK). The slides were photographed using a DP70 camera adapter system [27,28]. All staining procedures and image analysis were carried out in a blinded manner. Approximately 150 islets were analysed per group. Islet parameters were determined using the 'closed polygon' tool in Olympus Cell^{F} analysis software. For co-localisation studies, NPY expression in alpha/PP/somatostatin cells was determined by counting cells with NPY and glucagon/PP/somatostatin expression and expressed as % of total alpha/PP/somatostatin cells.

2.8 Biochemical analyses

Blood samples were collected from the cut tip on the tail vein of conscious mice at the time points indicated in Figure 3. Blood glucose was measured directly using a hand-held Ascencia Contour blood glucose meter (Bayer Healthcare, Newbury, Berkshire, UK). For plasma insulin analysis, blood samples were collected into chilled fluoride/heparin glucose micro-centrifuge tubes (Sarstedt, Numbrecht, Germany) and immediately centrifuged using a Beckman microcentrifuge (Beckman Instruments, Galway, Ireland) for 1 min at 13,000 x *g* and stored at -20 °C, prior to determination of insulin by a radioimmunoassay [24].

2.9 Statistical analysis

Statistical analyses were performed using GraphPad PRISM software (Version 5.0). Values are expressed as mean±S.E.M. Comparative analyses between groups were carried out using a One-way ANOVA with Berferroni post hoc test or student's unpaired t-test, as appropriate. The difference between groups was considered significant if $p<0.05$.

3. Results

3.1 Expression of NPY in isolated mouse islets and effects of NPY and PP on insulin release from cultured rodent and human beta-cells and isolated mouse islets

NPY mRNA was detected in mouse islets (n=5), but expression levels were significantly lower (0.03±0.01%; $p<0.001$) relative to the housekeeping β-actin gene, as was the expression of somatostatin (13.9±2.8%; $p<0.01$) and PP (6.9±0.2%; $p<0.001$), whereas glucagon expression (220.2±4.8%; $p<0.001$) was higher.

As expected, KCl (30 mM), alanine (10 mM) and GLP-1 (10^{-8} - 10^{-6} M) evoked significant ($p<0.05$ to $p<0.001$) insulin release from BRIN BD11 cells at both 5.6 and 16.7 mM glucose (Fig. 1A,B). These secretagogues also induced significant ($p<0.05$ to $p<0.001$) insulin secretion at 16.7 mM glucose concentrations from human 1.1B4 beta-cells (Fig. 1C). NPY and PP did not exert any significant effects on insulin secretion at 5.6 mM glucose in BRIN BD11 cells (Fig. 1A). However, at 16.7 mM glucose NPY significantly ($p<0.001$) inhibited insulin release at all concentrations examined, baring 10^{-10} M (Fig. 1B). In harmony, PP also significantly ($p<0.05$ to $p<0.001$) inhibited insulin release from BRIN BD11 cells at 16.7 mM glucose, but only at the highest peptide concentrations (10^{-6} and 10^{-7} M) tested (Fig. 1B). NPY and PP (10^{-6} M) similarly inhibited ($p<0.001$ and $p<0.05$, respectively) insulin release from 1.1B4 cells by 12 and 19%, respectively (Fig. 1C). However, NPY and PP concentrations of 10^{-8} M had no obvious effect on the insulin secretory function in this cell line (Fig. 1C).

Observations in rodent and human beta-cell lines (Fig. 1A-C) were largely in agreement with data derived from isolated mouse islets (Fig. 1D). As such, NPY (10^{-10} - 10^{-6} M) significantly inhibited ($p<0.001$) insulin release from mouse islets at 16.7 mM glucose (Fig. 1D). However, PP was without effect on insulin secretion from isolated mouse islets (Fig. 1D). Studies were conducted in BRIN BD11 cells using specific antagonists of NPYR1, NPYR2 or

NPYR5 to evaluate which NPY receptors are involved. Interestingly, incubation of 10^{-6} M NPY with the NPY1 specific receptor antagonist BVD-10 partially reversed the insulinostatic effect of this peptide at 16.7 mM glucose (Fig. 1E). Similar observations were made with both the NPY2 and NPY5 specific receptor antagonists BII0246 and CGP71983, respectively, that significantly ($p<0.001$) enhanced insulin secretion evoked by 10^{-6} M NPY at 16.7 mM glucose, but did not completely restore levels to that of control (Fig. 1E). In contrast, neither BVD-10, BII0246 nor CGP71983 had any significant effect on the insulinostatic effect of PP (Fig. 1E).

3.2 Effects of NPY and PP on membrane potential and intracellular Ca^{2+} levels in BRIN BD11 cells

BRIN BD11 cell membrane was depolarised and $(Ca^{2+})_i$ increased ($p<0.001$) by KCl (10 mM) and alanine (10 mM), respectively, at both 5.6 and 16.7 mM glucose (Fig. 2A-D). In contrast, neither NPY nor PP had any significant effect on membrane potential and $(Ca^{2+})_i$ when compared to respective glucose controls (Fig. 2A-D). A tendency towards hyperpolarisation was evident (Fig. 2A-D). Consistent with such action, alanine-induced elevations of membrane potential were partially reserved by both NPY and PP (Fig. 2E). In addition, elevations of $(Ca^{2+})_i$ stimulated by alanine were significantly inhibited by both NPY ($p<0.05$) and PP ($p<0.001$) in BRIN BD11 cells (Fig. 2F).

3.3 Effects of NPY and PP on glucose tolerance and feeding

NPY and PP were administered intraperitoneally at a dose of 25 nmol/kg in combination with glucose to normal mice. No significant effects were observed on individual glucose and insulin values over the 90 minute experimental period (Fig. 3A,C). However, there was a strong

tendency for both NPY and PP to reduce glucose-stimulated insulin concentrations, particularly at 15 min post-injection (Fig. 3C). Corresponding 0-60 min glucose and insulin AUC data were also not significantly different between the groups (Fig. 3B,D). However, whilst PP did not affect acute feeding behaviour in overnight fasted mice, NPY significantly ($p<0.05$ to $p<0.01$) increased food intake at 90, 150 and 180 min post-injection (Fig. 3E).

3.4 Effects of streptozotocin and hydrocortisone on islet expression of NPY and PP in C57BL/6 mice

Blood glucose and body weights of streptozotocin and hydrocortisone treated mouse were assessed on day 10 of the respective treatment interventions. As would be expected, streptozotocin mice had significantly ($p<0.001$) elevated blood glucose levels compared to saline controls (21.0 ± 2.1 *vs.* 7.2 ± 0.5 mmol/l; respectively), whereas blood glucose concentrations in hydrocortisone mice were comparable to controls (6.2 ± 0.2 mmol/l). Similarly, hydrocortisone treatment for 10 days did not significantly affect body weight compared to saline control mice (32.2 ± 0.3 *vs.* 24.2 ± 0.4 g; respectively), whilst streptozotocin treatment resulted in significantly ($p<0.05$) reduced body weight (22.6 ± 0.3 g). Figure 4A depicts pancreatic islets from saline, streptozotocin and hydrocortisone treated mice. As previously established, streptozotocin reduced ($p<0.01$), and hydrocortisone increased ($p<0.001$), beta-cell area (data not shown). The reduction of beta-cell area in streptozotocin mice was partly offset by increases ($p<0.001$) in alpha cell areas, whereas hydrocortisone-treated mice had similar alpha cell areas when compared to control mice (data not shown). Neither treatment regimen affected delta cell area. PP cell area was similar in control and hydrocortisone treated mice at 1918 ± 195 and 3954 ± 412 μm^2, respectively, but significantly ($p<0.001$) increased (3954 ± 412.3 μm^2) in streptozotocin mice. Islet images of fluorescently

stained pancreata from control, streptozotocin and hydrocortisone treated mice showing PP location, and co-localisation of NPY with glucagon, PP or somatostatin are presented in Figure 4B-E. Whilst PP staining appeared to be elevated in streptozotocin mice in a similar fashion to glucagon, it remained relatively unaltered in hydrocortisone mice (Fig. 4B). It was also clear that exocrine NPY expression (red staining) was increased under the cellular stresses induced by both streptozotocin and hydrocortisone treatment (Fig. 4C-E). There was very little co-localisation of NPY with glucagon in all three groups of mice (Fig. 4C,F). Interestingly, in islets NPY appeared to be mainly co-localised with somatostatin in streptozotocin treated mice (Fig. 4D). This was confirmed by quantitative analysis of NPY co-localisation with somatostatin and PP in these mice (Fig. 4G,H). As such, hydrocortisone treatment did not alter normal co-localisation of NPY with PP (Fig. 4G,H), whereas streptozotocin significantly ($p<0.05$) increased NPY co-localisation with somatostatin (Fig. 4G) and decreased ($p<0.05$) co-localisation with PP (Fig. 4H).

3.5 Effects of NPY and PP on rodent BRIN BD11 and human 1.1B4 beta-cell proliferation

Culture of rodent BRIN BD11 or human 1.1B4 beta-cells in the presence of GLP-1 (10^{-6} M) for 16 h significantly ($p<0.001$ to $p<0.01$; respectively) increased proliferation frequency when compared to control cultures (Fig. 5A,B). NPY (10^{-6} M) similarly increased ($p<0.01$) cellular proliferation frequency in BRIN BD11 cells (Fig. 5A), but this was not apparent in human 1.1B4 cells (Fig. 5B). PP (10^{-6} M) did not alter proliferation in BRIN BD11 or 1.1B4 beta-cells when compared to control cultures (Fig. 5A,B). Figure 5C shows representative images of Ki67 stained BRIN BD11 and 1.1B4 beta-cells under each culture condition.

3.6 Protective effects of NPY and PP on streptozotocin-induced DNA damage in rodent BRIN BD11 and human 1.1B4 beta-cells

Streptozotocin (5 mM) significantly ($p<0.001$) decreased cell viability in BRIN BD11 cells which was fully reversed by GLP-1, NPY and PP treatment (Fig. 6A). Similar effects were also noted in 1.1B4 cells, although the benefits of NPY or PP were not as striking, and cell viability was still significantly ($p<0.01$ to $p<0.001$) reduced compared to control cultures (Fig. 6B). In a similar fashion, percentage tail DNA was significantly ($p<0.001$) increased by streptozotocin treatment in BRIN BD11 cells, but fully reversed by both NPY and PP (Fig. 6C). This was also the case for NPY in 1.1B4 cells, but % tail DNA was still partially ($p<0.001$) elevated in PP treated cells following streptozotocin insult (Fig. 6D). Olive tail moment, defined as the product of the tail length and the fraction of total DNA in the tail, was significantly ($p<0.001$) increased in BRIN BD11 and 1.1B4 cell lines following streptozotocin treatment (Fig. 6E,F). Whilst both NPY and PP protected against this, olive tail moment was still significantly ($p<0.001$) elevated compared to control cultures (Fig. 6E,F). Figure 6G shows representative comet images from each culture condition in BRIN BD11 and 1.1B4 beta-cells.

4. Discussion

The perception that non-classical islet peptide hormones have an important role to play in the regulation of pancreatic endocrine function is gaining more acceptance. For example, ghrelin is now recognised as a genuine pancreatic islet hormone involved in insulin secretion and glucose homeostasis [29]. Moreover, there is emerging evidence of a modulatory role for glucagon-like peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), cholecystokinin (CCK) and PYY, amongst others, in the local paracrine control beta-cell mass and insulin secretion [4,26,28,30,31], especially in situations of metabolic stress as encountered in

diabetes. We have previously confirmed expression of all main NPYR isoforms in mouse pancreatic islets, including NPYR1, 2, 4 and 5 [4]. While others suggest limited to no expression of NPYR2 in human pancreatic islets [32], we observed low, but detectable, NPYR2 expression in primary mouse islets, as well as both human and rodent beta-cell lines [4]. Taken together, this would suggest a role of intra-islet NPYRs in the regulation endocrine pancreatic function.

NPY and PP share a common tertiary structure motif and exhibit 50% homology [1], and it is thus unsurprising that both peptides bind to, and interact with, NPYRs [8]. Despite their structural similarity, NPY shows clear specificity for NPYR1, 2 and 5, whereas PP is considered to be a preferential ligand for NPYR4 [9]. Indeed, our studies with selective NPY1, 2 and 5 receptor antagonists fully support this. Neither NPY nor PP affected insulin secretion at non-stimulatory basal glucose levels, but both peptides prominently inhibited GSIS from rodent and human beta-cell lines, in line with previous observations for NPY [18]. The action of NPY, but not PP, was partially reversed by each of the specific NPYR1, 2 and 5 antagonists, BVD-10, BIIE 0246 and CGP71683. The insulinostatic effect of PP was less obvious than NPY in beta-cell lines, and was completely absent in isolated mouse islets. This difference is intriguing, especially since NYPR4 expression is particularly prominent in islets [4], suggesting important paracrine interactions. In this regard, PP has recently been shown to inhibit somatostatin secretion [33], which could certainly help explain the current findings. In association with this, a separate study employing triple deletion of NPYR1, 2 and 4 in mice, has highlighted their combined key role in the regulation of circulating insulin levels [34]. Furthermore, the inhibitory effect of NPY, PP and PYY on insulin secretion was previously shown to be relatively comparable [35-37], and although in slight disagreement with our observations, does suggest potentially similar mechanisms of action on beta-cells. In our investigations we detected no significant effect of NPY and PP on BRIN BD11 beta-cell membrane potential and

$(Ca^{2+})_{i,}$, but both peptides had a tendency to induce transient membrane hyperpolarisation, especially at high glucose concentrations. Consistent with this, NPY and PP inhibited of alanine-induced changes of these parameters, broadly mirroring observations made with PYY [4].

As expected [38], NPY significantly increased food intake in overnight fasted mice, whereas PP was devoid of appetite-suppressive actions at the dose employed. Recent studies would suggest a possible anorexigenic action of PP [39], but this appears to be due to stimulation of sensory neurons following release of PP from F cells. Whilst others have shown inhibition of food intake in mice following acute administration of PP, this has been observed at doses approximately double the 25 nmol/kg utilised in the current study [40]. Indeed, similar to our findings, no appetite suppressive effects of PP have been observed at a dose of 10 nmol/kg, and inhibitory effects of 30 mmol/kg were only apparent over 3 hours after administration [41], which is well beyond the observation period employed in the current setting. In agreement with knowledge that NPY overexpressing transgenic mice do not present with altered glucose homeostasis or GSIS [42], a bolus administration of either NPY or PP in combination with glucose had no impact on GSIS or circulating glucose in mice. Interestingly, NPY deficient mice have mildly increased basal and glucose stimulated insulin concentrations [43]. The difference between *in vitro* and *in vivo* observations is likely related to specific concentrations of peptides exposed to cells, and lack of opportunity for neuronal and/or local paracrine islet cell communication in the *in vitro* setting [33]. Expression of NPY in islets from normal healthy mice was lower than the classical islets hormones glucagon, somatostatin and PP. This likely reflects the fact that NPY is also known to be located in the sympathetic nerve fibres within the pancreas, exerting inhibiting tone on insulin secretion [44].

Pancreatic expression of NPY has been shown to altered in diabetes [15], suggesting a possible involvement in the maintenance of glucose homeostasis. In agreement, following

treatment of mice with streptozotocin and development of insulin-deficient diabetes, endocrine NPY expression changed from co-localisation with PP cells to more pronounced co-expression with delta-cells, similar to observations in high fat fed diabetic rats [15]. This alteration in expression was not observed in hydrocortisone treated mice, and could be linked to the less severe form of diabetes induced by this treatment intervention. Thus, the influence of NPY originating from islet cells is likely to be paracrine in nature, closely related to somatostatin secretion or action [45], with an important role in cellular adaptations to beta-cell destruction that would be encountered in certain forms of diabetes. Interestingly, we also observed up-regulation of NPY expression in exocrine nerve endings following both streptozotocin and hydrocortisone intervention, implying key adaptive functions in situations of both insulin deficiency and resistance provoked diabetes. Taken together, these observations suggest that activation of NPYRs by NPY and PP have a potentially important impact on beta-cell survival and function, and the islet adaptations that occur either in the development, or as a result of, diabetes. Further studies would be required to fully delineate the role of NPY and PP mediated islet signalling in the progression of diabetes, but our studies indicate important alterations of intra-islet actions of both hormones. To further probe this hypothesis, we conducted more detailed studies to assess the proliferative and anti-apoptotic actions of NPY and PP in cultured rodent and human beta-cells.

In BRIN BD11 cells, NPY significantly augmented beta-cell proliferation, but this positive effect was not apparent in human 1.1B4 cells. In contrast, PP did not evoke enhanced proliferative responses in either cell line. NPY1 expression was shown to be particularly abundant in BRIN BD11 cells [4], which may help explain these findings. Indeed, NPYR1 activation is recognised to exert cell growth factor properties through elevated extracellular signal-regulated kinase activity [19,46]. There is a suggestion that activation of NPYRs protects against beta-cell loss by preventing apoptosis, rather than having direct proliferative effects

[47]. In agreement, both NPY and PP fully protected BRIN BD11, and partially protected 1.1B4, beta-cells from streptozotocin-induced beta-cell death, indicating involvement of all major NPYRs in this beneficial effect. Our previous studies revealed that the NPYR agonists, PYY(1-36) and PYY(3-36), also exhibited pronounced protective effects against streptozotocin-induced beta-cell DNA damage [4]. NPY appeared to be somewhat more effective than PP, indicating less prominent involvement of NPYR4s in this regard. The ability of NPY to inhibit apoptosis has been linked to increased PKC epsilon activity [48], and would be interesting to assess levels of this enzyme in future studies, to help clarify anti-apoptotic efficacy of both peptides. Interestingly, a study in transgenic mice overexpressing NPY under the rat insulin promoter gene revealed no impact of NPY on beta-cell area, despite islet NPY levels being 25 times higher than normal [42]. Effects on beta-cell proliferation and apoptosis were not assessed in this study and these observations could simply highlight the plasticity of pathways involved in the regulation of beta-cell mass [5]. Moreover, down regulation of NPYRs has been evidenced in the face of chronically elevated NPY concentrations [49], which could also have a major impact on findings from NPY overexpressing transgenic mice [42].

In conclusion, PP, and particularly NPY, have important metabolic actions linked to altered beta-cell secretory function, that appear to ultimately aid in the preservation of insulin secretion and beta-cell mass. We also observed modified NPY and PP intra-islet expression in response to diabetic milieu induced by both insulin deficiency and resistance, highlighting possible important islet adaptive functions of both peptides. Taken together, these data suggest that direct modulation of the NPY family of receptors could prove to be an important therapeutic target for diabetes.

Acknowledgements

The study was supported in part by an EFSD/Boehringer Ingelheim grant, donation from the SAAD Trading and Contracting Company and award of an Ulster University Vice Chancellor's research scholarship to DK.

Disclosure

The authors declare that no conflicting interests exist.

References

1. Brothers SP, Wahlestedt C. Therapeutic potential of neuropeptide Y (NPY) receptor ligands. Embo Molecular Medicine 2 (2010) 429-439.
2. Loh K, Herzog H, Shi YC. Regulation of energy homeostasis by the NPY system. *Trends in* Endocrinology and Metabolism 26 (2015) 125-35.
3. Goldstone AP. The hypothalamus, hormones, and hunger: alterations in human obesity and illness. Progress in Brain Research 153 (2006) 57-73.
4. Khan D, Vasu S, Moffett RC, Irwin N, Flatt PR. Islet distribution of Peptide YY and its regulatory role in rodent and human beta-cell function and survival. Molecular and Cellular Endocrinology 436 (2016) 102-113.

5. Persaud SJ, Bewick GA. Peptide YY: More than just an appetite regulator. Diabetologia 57 (2014) 1762-1769.
6. Shi YC, Loh K, Bensellam M. *et al*. Pancreatic PYY Is Critical in the Control of Insulin Secretion and Glucose Homeostasis in Female Mice. Endocrinology 156 (2015) 3122-36.
7. Ramracheya RD, McCulloch LJ, Clark A *et al*. PYY-Dependent Restoration of Impaired Insulin and Glucagon Secretion in Type 2 Diabetes following Roux-En-Y Gastric Bypass Surgery. Cell Reports 15 (2016) 944-50.
8. Lin S, Boey D, Herzog H 2004 NPY and Y receptors: lessons from transgenic and knockout models. Neuropeptides 38 (2004) 189-200.
9. Alexander SP, Benson HE, Faccenda E, Pawson AJ, Sharman JL, Spedding M, *et al*. The Concise Guide to PHARMACOLOGY 2013/14: G protein-coupled receptors. British Journal of Pharmacology 170 (2013) 1459–1581.
10. Amisten S, Salehi A, Rorsman P, Jones PM, Persaud SJ. An atlas and functional analysis of G-protein coupled receptors in human islets of langerhans. Pharmacology & Therapeutics 139 (2013) 359-391.
11. Ding WG, Fujimura M, Mori A, Tooyama I, Kimura H. Light and electron-microscopy of neuropeptide-Y containing nerves in human liver, gallbladder, and pancreas. Gastroenterology 101 (1991) 1054-1059.
12. Myrsen U, Ahren B, Sundler F. Neuropeptide-Y is expressed in subpopulations of insulin-producing and non-insulin-producing islet cells in the rat after dexamethasone treatment - a combined immunocytochemical and in-situ hybridization study. Regulatory Peptides 60 (1995) 19-31.

13. Waeber G, Thompson N, Waeber B, Brunner HR, Nicod P, Grouzmann E. Neuropeptide-Y expression and regulation in a differentiated rat insulin-secreting cell-line. Endocrinology 133 (1993) 1061-1067.
14. Ahrén B, Wierup N, Sundler F. Neuropeptides and the Regulation of Islet Function. Diabetes 55 (2006) S98-S107.
15. Ruipan Z, Xiangzhi M, Li L, Ying Z, Mingliang Q, Peng J, Jingwei L, Zijun Z, Yan G. Differential expression and localization of neuropeptide Y peptide in pancreatic islet of diabetic and high fat fed rats. Peptides 54 (2014) 33-8.
16. Whim MD. Pancreatic beta cells synthesize neuropeptide Y and can rapidly release peptide co-transmitters. Plos One 6 (2011) e19478.
17. Ekblad E, Sundler F. Distribution of pancreatic polypeptide and peptide YY. Peptides 23 (2002) 251-261.
18. Moltz JH, McDonald JK. Neuropeptide Y: direct and indirect action on insulin secretion in the rat. Peptides 6 (1985) 1155-9.
19. Cho YR, Kim CW. Neuropeptide Y promotes beta-cell replication via extracellular signal-regulated kinase activation. Biochemical and Biophysical Research Communications 314 (2004) 773-780.
20. Pathak V, Vasu S, Gault VA, Flatt PR, Irwin N. Sequential induction of beta cell rest and stimulation using stable GIP inhibitor and GLP-1 mimetic peptides improves metabolic control in C57BL/KsJ db/db mice. Diabetologia 58 (2015) 2144-2153.
21. McClenaghan NH, Barnett CR, Ah-Sing E, Abdel-Wahab YH, O'Harte FP, Yoon TW, Swanston-Flatt SK, Flatt PR. Characterization of a novel glucose-responsive insulin-secreting cell line, BRIN-BD11, produced by electrofusion. Diabetes 45 (1996) 1132-1140.

22. McCluskey JT, Hamid M, Guo-Parke H, McClenaghan NH, Gomis R, Flatt PR. Development and functional characterization of insulin-releasing human pancreatic beta cell lines produced by electrofusion. Journal of Biological Chemistry 286 (2011) 21982-21992.
23. Hamid M, McCluskey JT, McClenaghan NH, Flatt PR. Comparison of the secretory properties of four insulin-secreting cell lines. Endocrine Research 28 (2002) 35-47.
24. Flatt PR, Bailey CJ. Plasma glucose and insulin response to glucagon and arginine in Aston ob/ob mice: evidence for a selective defect in glucose-mediated insulin release. Hormone and Metabolic Research 14 (1982) 127-30.
25. Miguel JC, Patterson S, Abdel-Wahab YH, Mathias PC, Flatt PR. Time-correlation between membrane depolarization and intracellular calcium in insulin secreting BRIN-BD11 cells: Studies using FLIPR. Cell Calcium 36 (2004) 43-50.
26. Lees Murdock DJ, Barnett YA, Barnett CR. DNA damage and cytotoxicity in pancreatic beta-cells expressing human CYP2E1. Biochemical Pharmacology 68 (2004) 523-530.
27. Moffett RC, Irwin N, Francis JME, Flatt PR. Alterations of Glucose-Dependent Insulinotropic Polypeptide and Expression of Genes Involved in Mammary Gland and Adipose Tissue Lipid Metabolism during Pregnancy and Lactation. PLoS ONE 8 (2013) e78560.
28. Vasu S, Moffett RC, Thorens B, Flatt PR. Role of endogenous GLP-1 and GIP in beta-cell compensatory responses to insulin resistance and cellular stress. PloS ONE 9 (2014) e101005.
29. Yada T, Damdindorj B, Rita R. *et al*. Ghrelin signalling in β-cells regulates insulin secretion and blood glucose. Diabetes Obesity and Metabolism 16 (2014) 111-117.

30. Lavine JA, Kibbe CR, Baan M, Sirinvaravong S, Umhoefer HM, Engler KA, Meske LM, Sacotte KA, Erhardt DP, Davis DB. Cholecystokinin expression in the β-cell leads to increased β-cell area in aged mice and protects from streptozotocin-induced diabetes and apoptosis. American Journal of Physiology Endocrinology and Metabolism 309 (2015) 819-28.
31. Moffett RC, Vasu S, Flatt PR. Functional GIP receptors play a major role in islet compensatory response to high fat feeding in mice. Biochimica et Biophysica Acta 1850 (2015) 1206-14.
32. Chandarana K, Gelegen C, Irvine EE, *et al*. Peripheral activation of the Y2-receptor promotes secretion of GLP-1 and improves glucose tolerance. Molecular Metabolism 2 (2013) 142-152.
33. Kim W, Fiori JL, Shin YK, Okun E, Kim JS, Rapp PR, Egan JM. Pancreatic polypeptide inhibits somatostatin secretion. FEBS Letters 588 (2014) 3233-9.
34. Lin ED, Sainsbury A, Lee NJ, Boey D, Couzens M, Enriquez R, Slack K, Bland R, During MJ, Herzog H. Combined deletion of Y1, Y2, and Y4 receptors prevents hypothalamic neuropeptide Y overexpression-induced hyperinsulinemia despite persistence of hyperphagia and obesity. Endocrinology 147 (2006) 5094-5101.
35. Ahren B, Larsson H. Plasma neuropeptide Y in impaired glucose tolerance. Acta Diabetologica 33 (1996) 295-297.
36. Bertrand G, Gross R, Roye M, Ahren B, Ribes G. Evidence for a direct inhibitory effect of PYY on insulin-secretion in rats. Pancreas 7 (1992) 595-600.
37. Park HJ, Lee YL, Kwon HY. Effects of pancreatic-polypeptide on insulin action in exocrine secretion of isolated rat pancreas. Journal of Physiology-London 463 (1993) 421-429.

38. Morley JE, Hernandez EN, Flood JF. Neuropeptide Y increases food intake in mice. American Journal of Physiology 253 (1987) R516-22.

39. Khandekar N, Berning BA, Sainsbury A, Lin S. The role of pancreatic polypeptide in the regulation of energy homeostasis. Molecular and Cellular Endocrinology 418 (2015) 33-41.

40. Machida Y, Bruinsma C, Hallinger DR, Roper SM, Garcia E, Trevino MB, Nadler J, Ahima R, Imai Y. Pancreatic islet neuropeptide Y overexpression has minimal effect on islet morphology and beta-cell adaptation to high-fat diet. Endocrinology 155 (2014) 4634-4640.

41. Liu YL, Semjonous NM, Murphy KG, Ghatei MA, Bloom SR. The effects of pancreatic polypeptide on locomotor activity and food intake in mice. International Journal of Obesity (London) 32 (2008) 1712-5.

42. Shi YC, Lin Z, Lau J, Zhang H, Yagi M, Kanzler I, Sainsbury A, Herzog H, Lin S. PYY3-36 and pancreatic polypeptide reduce food intake in an additive manner via distinct hypothalamic dependent pathways in mice. Obesity (Silver Spring) 21 (2013) E669-78.

43. Imai Y, Patel HR, Hawkins EJ, Doliba NM, Matschinsky FM, Ahima RS. Insulin secretion is increased in pancreatic islets of neuropeptide Y-deficient mice. Endocrinology 148 (2007) 5716-23.

44. Myrsen U, Ahren B, Sundler F. Dexamethasone-induced neuropeptide Y expression in rat islet endocrine cells - rapid reversibility and partial prevention by insulin. Diabetes 45 (1996) 1306-1316.

45. Myrsén U, Sundler F. Neuropeptide Y is expressed in islet somatostatin cells of the hamster pancreas: a combined immunocytochemical and in situ hybridization study. Regulatory Peptides 57 (1995) 65-76.

46. Hansel DE, Eipper BA, Ronnett GV. Neuropeptide Y functions as a neuroproliferative factor. Nature 410 (2001) 940-944.

47. Sam AH, Gunner DJ, King A, Persaud SJ, Brooks L, Hostomska K, Ford HE, Liu B, Ghatei MA, Bloom SR, Bewick GA. Selective ablation of peptide YY cells in adult mice reveals their role in beta-cell survival. Gastroenterology 143 (2012) 459-468.

48. Mannon PJ. Peptide YY as a growth factor for intestinal epithelium. Peptides 23 (2002) 383-388.

49. Widdowson PS. Regionally-selective down-regulation of NPY receptor subtypes in the obese Zucker rat. Relationship to the Y5 'feeding' receptor. Brain Research 758 (1997) 17-25.

Target	Host	Dilution	Source

Table 1. Target, host and source of primary and secondary antibodies employed for immunoflourescent islet histology studies

Primary antibodies

Insulin	Mouse	1:500	Abcam, ab6995
Glucagon	Guinea pig	1:200	Raised in-house PCA2/4
NPY	Mouse	1:500	Abcam, ab112473
PP	Goat	1:200	Abcam, ab77192
SST	Rat	1:500	Biorad, 8330-009
Ki67	Rabbit	1:200	Abcam, ab15580

Secondary antibodies

Target	**Host**	**Reactivity**	**Dilution**	**Fluorescent dye and Source**
IgG	Goat	Mouse	1:400	Alexa Flour 594, Invitrogen, UK
IgG	Goat	Guinea pig	1:400	Alexa Flour 488, Invitrogen, UK
IgG	Goat	Rabbit	1:400	Alexa Flour 594, Invitrogen, UK
IgG	Goat	Rat	1:400	Alexa Flour 488, Abcam
IgG	Donkey	Goat	1:400	Alexa Flour 488, Invitrogen, UK

Figure Legends

Fig. 1. Effects of NPY and PP on insulin release from rodent BRIN BD11 beta-cells, cultured human 1.1B4 beta-cells and isolated mouse islets. (A,B) BRIN BD11, (C) 1.1B4 cells (D) or isolated mouse islets were incubated with either 5.6 or 16.7 mM glucose, as appropriate, and the effects of NPY or PP (10^{-10} – 10^{-6} M) determined. (E) Effects of selective NPY1 (BVD-10), NPY2 (BII0246) and NPY5 (CGP71683) receptor antagonists on NPY and PP modulation of insulin release from rodent BRIN BD11 beta-cells. Values are mean ± SEM (A-C,E n=8; D n=4). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ compared to respective glucose alone control. $^{\Delta\Delta\Delta}p<0.001$ compared to NPY 10^{-6} M.

Fig. 2. Effects of NPY and PP on membrane potential and $(Ca^{2+})_i$ in rodent BRIN BD11 cells. Cells were incubated with (A,B) 5.6 or (C,D) 16.7 mM glucose and NPY or PP (both at 10^{-6} M), or the positive controls alanine and KCl (both at 10 mM) and (A,C) membrane potential or (B,D) $(Ca^{2+})_i$ were assessed over a 5 minute period. (E,F) Cells were incubated with 5.6 mM glucose and NPY or PP (both at 10^{-6} M) in the absence or presence of alanine (10 mM) and (E) membrane potential or (F) $(Ca^{2+})_i$ assessed over a 5 minute period. (A-F) Area under curve data is also shown. Values are mean ± SEM (n=6). $^{***}p<0.001$ compared to respective glucose control. $^{\Delta}p<0.05$, $^{\Delta\Delta\Delta}p<0.001$ compared to respective positive control.

Fig. 3. Acute effects of NPY and PP on glucose tolerance, insulin response to glucose and food intake in overnight fasted mice. (A) Blood glucose and (B) plasma insulin levels were assessed immediately before and after intraperitoneal administration of NPY or PP (25 nmol/kg bw) together with glucose (18 mmol/kg bw). Respective (C) blood glucose and (D) plasma insulin area under curve data is also shown. (E) Cumulative food intake was assessed after intraperitoneal administration of saline vehicle (0.9% NaCl), NPY or PP (25 nmol/kg

bw) in overnight fasted mice Values are mean ± SEM (n=6 mice). $^{*}p<0.05$, $^{**}p<0.01$ compared to saline treated mice.

Fig. 4. Effects of streptozotocin and hydrocortisone treatment of mice on islet insulin and glucagon staining, PP localisation and co-localisation of NPY with glucagon, PP and somatostatin. C57BL/6 mice (n=6) received daily injections of streptozotocin (50 mg/kg bw) or hydrocortisone (70 mg/kg bw) for 5 or 10 days respectively before examination of pancreatic histology on day 10. Representative islet images showing (A) insulin (red) and glucagon (green) staining, (B) PP (red) staining, and NPY (red) with (C) glucagon, (D) PP and (E) somatostatin (green) in islets of control, streptozotocin and hydrocortisone treated mice are shown. Nuclei are demonstrated using DAPI staining (blue). Arrows indicate (B) cells that are positive for PP, (D) cells that are positive for both NPY and somatostatin and (E) cells that are positive for both NPY and PP. Quantification of co-localisation of NPY with (F) glucagon, (G) somatostatin and (H) PP is also shown. Values are mean ± SEM (n=6 mice). $^{*}p<0.05$ compared to control mice.

Fig. 5. Effects of NPY and PP on rodent BRIN BD11 and cultured human 1.1B4 cell proliferation. (A,B) Proliferation frequency in (A) BRIN BD11 and (B) 1.1B4 cells cultured with GLP-1, NPY and PP (all at 10^{-6} M) for 16 h. (C) Representative images showing proliferating beta-cells in the presence (16 h) of GLP-1, NPY and PP. Arrows indicate proliferating cells. Values are mean ± SEM (n=4). $^{**}p<0.01$, $^{***}p<0.001$ compared to control cultures.

Fig. 6. Effects of NPY and PP on protection of rodent BRIN BD11 cells and cultured human 1.1B4 cell from streptozotocin induced cellular stress. (A,B) Cell viability, (C,D) % tail DNA and (E,F) olive tail moment were assessed in response to 16 h exposure to 5 mM streptozotocin with and without co-culture with GLP-1, NPY and PP (all at 10^{-6} M) in (A,C,E) BRIN BD11 and (B,D,F) 1.1B4 beta-cells. (G) Representative images showing comets of control, streptozotocin alone and in combination with NPY and PP (10^{-6} M) in both cell types. Arrows indicate cells with comet tails. Values are mean ± SEM (n=4). $^{*}p<0.015$, $^{**}p<0.01$, $^{***}p<0.001$ compared to control cultures. $^{\Delta\Delta\Delta}p<0.001$ compared to STZ (5 mM) alone.

Figure 1

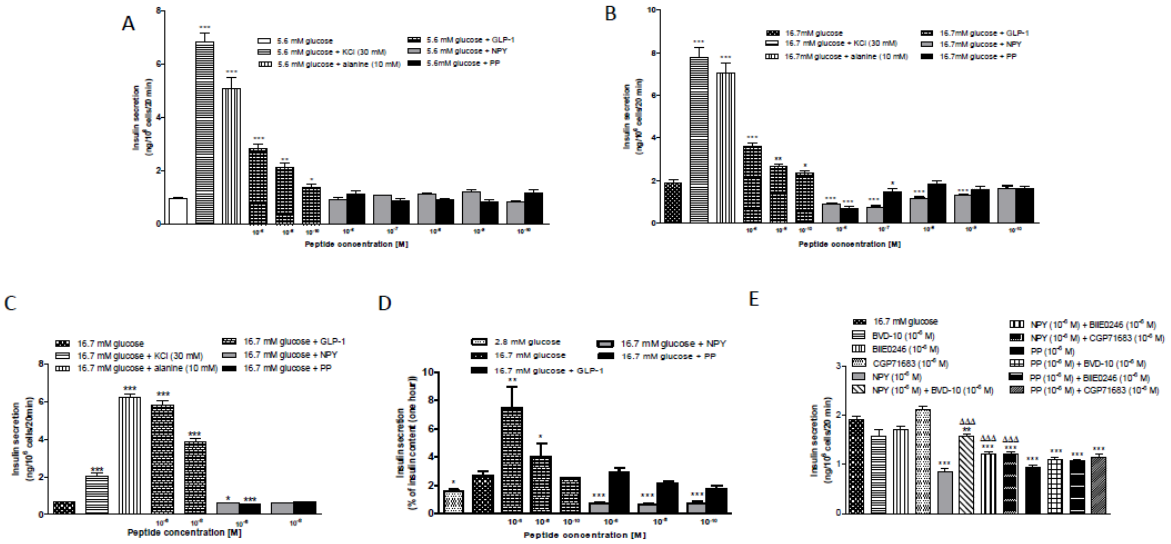


Figure 2

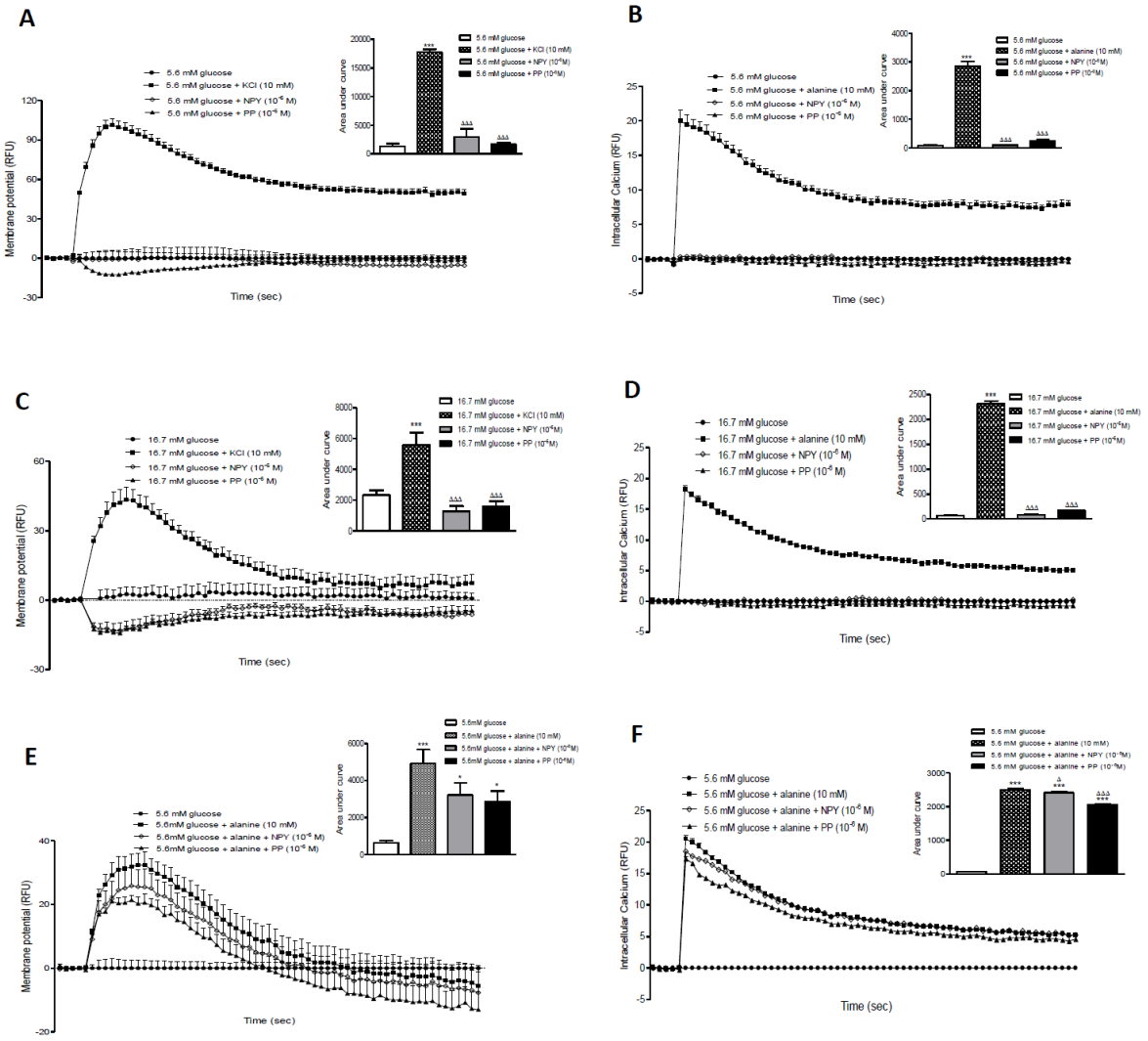


Figure 3

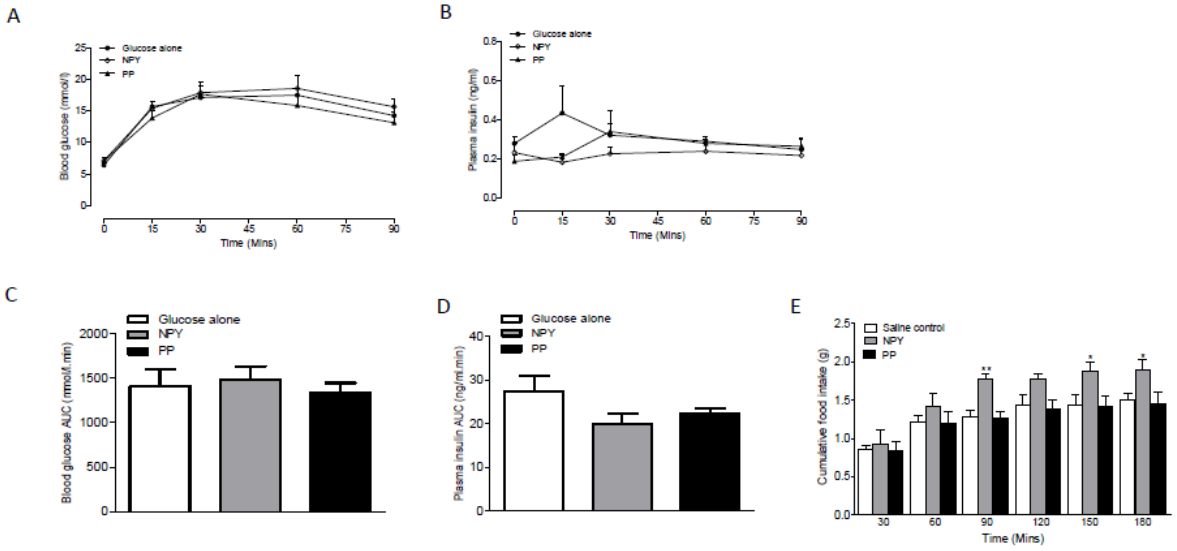


Figure 4

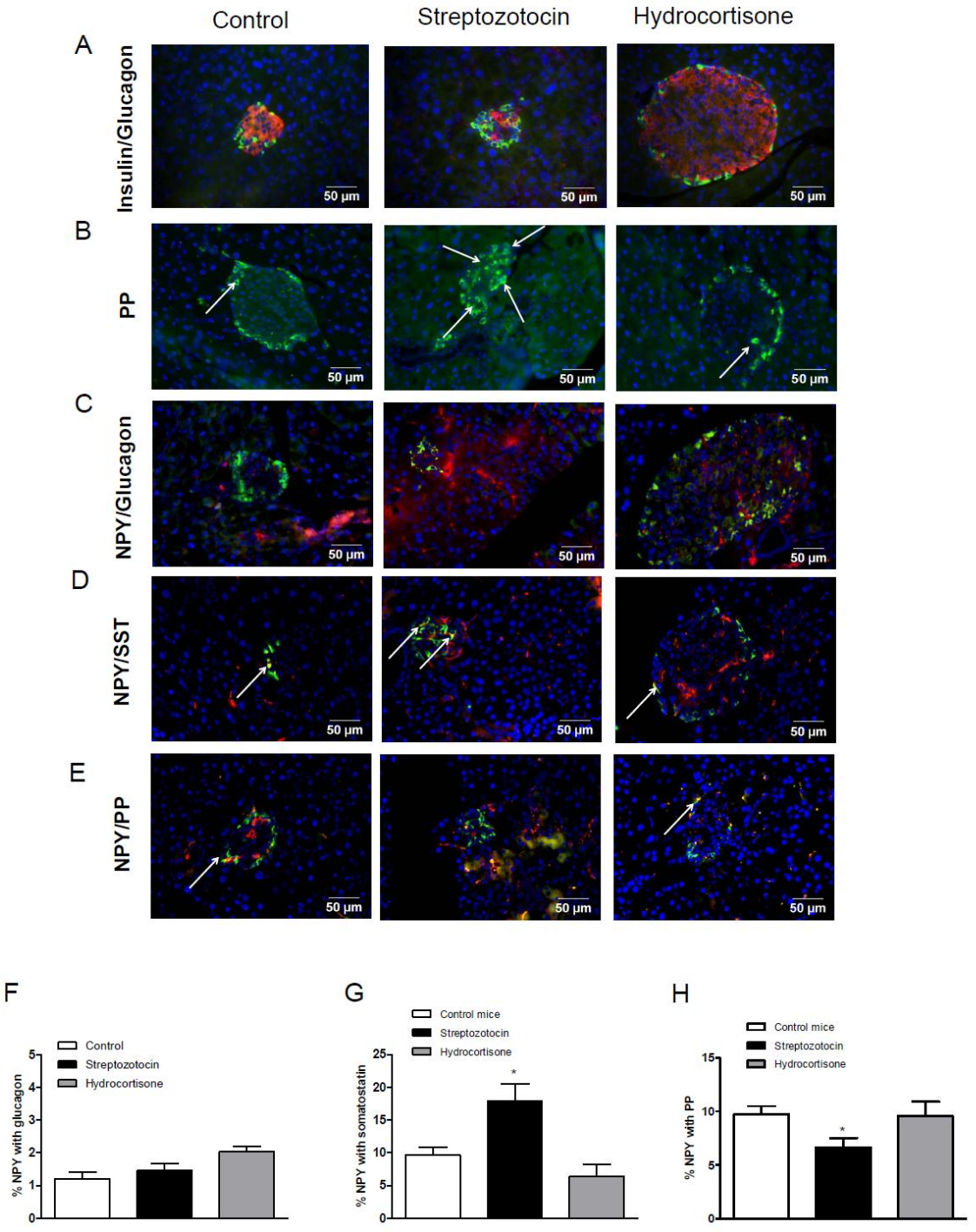


Figure 5

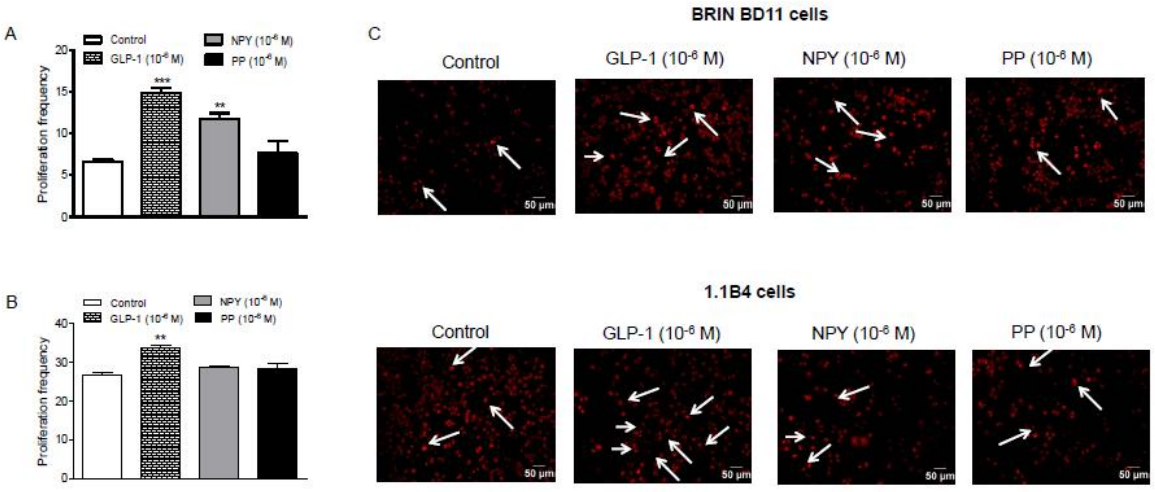
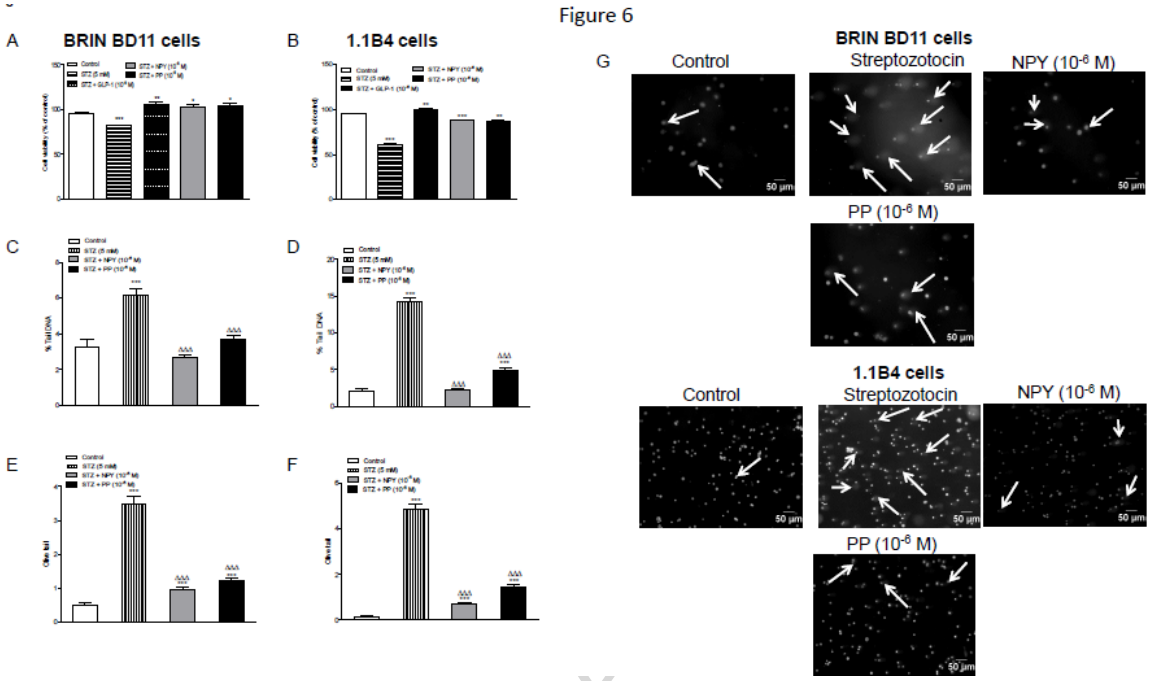


Figure 6



Highlights

- Evidence suggests neuropeptide Y (NPY) signaling helps preserve pancreatic beta-cell mass
- NPY and pancreatic polypeptide (PP) inhibit glucose-stimulated insulin secretion
- NPY and PP exerted protective effects against beta-cell apoptosis
- NPY augments beta-cell proliferation
- Diabetes alters the expression and islet localization of NPY and PP