Neuropeptide B stimulates insulin secretion and expression but not proliferation in rat insulin-producing INS-1E cells

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Abstract. Neuropeptide B (NPB) regulates food intake, body weight and energy homeostasis by interacting with NPBW1/NPBW2 in humans and NPBW1 in rodents. NPB and NPBW1 are widely expressed in the central nervous system and peripheral tissues including pancreatic islets. Although previous studies have demonstrated a prominent role for NPB and NPBW1 in controlling glucose and energy homeostasis, it remains unknown as to whether NPB modulates pancreatic β-cell functions. Therefore, the aim of the present study was to investigate the effects of NPB on insulin expression and secretion *in vitro*. Furthermore, the role of NPB in the modulation of INS-1E cell growth, viability and death was examined. Gene expression was assessed by reverse transcription-quantitative PCR. Cell proliferation and viability were determined by BrdU or MTT tests, respectively. Apoptotic cell death was evaluated by relative quantification histone-complexed DNA fragments (mono-and oligonucleosomes). Insulin secretion was studied using an ELISA test. Protein phosphorylation was assessed by western blot analysis. NPB and NPBW1 mRNA was expressed in INS-1E cells and rat pancreatic islets. In INS-1E cells, NPB enhanced insulin 1 mRNA expression via an ERK1/2-dependent mechanism. Furthermore, NPB stimulated insulin secretion from INS-1E cells and rat pancreatic islets. By contrast, NPB failed to affect INS-1E cell growth or death. We conclude that NPB may regulate insulin secretion and expression in INS-1E cells and insulin secretion in rat pancreatic islets.

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Abbreviations: BrdU, 5-bromo-2'-deoxyuridine; ERK1/2, extracellular signal-regulated protein kinases 1 and 2; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; Gck, glucokinase; Glut2, glucose transporter 2; GPCR, G protein-coupled receptor; Hnf4α, hepatocyte nuclear factor 4α; Hprt, hypoxanthine-guanine phosphoribosyltransferase; Mafa, β-cell transcriptional factor musculoaponeurotic fibrosarcoma oncogene family A; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; NPB, neuropeptide B; NPBW1, neuropeptides B/W receptor 1, GPR7; NPBW2, neuropeptides B/W receptor 2, GPR8; Pdx1, pancreatic and duodenal homeobox 1; Pgc1α, peroxisome proliferator-activated receptor γ coactivator 1-α

Key words: β-cells, INS-1E, insulin, neuropeptide B, pancreatic islets, proliferation

Introduction

Neuropeptide B is a 29 amino acid peptide with a C-6-brominated tryptophan residue at the N terminus (1,2). Its biological activities are mediated by activation of two GPCR receptors, termed NPBW1 (GPR7) and NPBW2 (GPR8). Both types of receptors are expressed in humans, whereas rodents only express NPBW1 (3). NPB and its receptors are predominantly expressed in the central nervous system (3). In the brain, NPB is implicated in controlling a variety of functions, including modulation of the neuroendocrine axis, pain, appetite or circadian rhythm (4-7). However, NPB and its receptor are also present in peripheral tissues such as thyroid and adrenal glands, gonads and endocrine pancreas (8). There is growing evidence that NPB as well as NPBW1 play prominent roles in controlling energy homeostasis. Animal studies have shown that mice lacking NPBW1 (NPBW1-/-) develop mild adult onset obesity, and have lower energy expenditure and higher blood glucose levels (9). Furthermore, increased body weight has been reported in mice lacking NPB (NPB-/-mice) (10). We found that NPB and its receptor are present in rat adipocytes (11) where NPB stimulates lipolysis and suppresses leptin expression, and secretion (11). Others have reported that NPB serum level is upregulated in humans who suffer from anorexia nervosa (12), while it is reduced in type 1 diabetic patients (13). Overall, these results collectively indicate that the NPB/NPBW1 system is involved in controlling body weight and energy homeostasis and its alternation may contribute to obesity.

Energy homeostasis and metabolism are modulated by insulin which is released from pancreatic beta cells in a

glucose-dependent fashion (14). The loss and dysfunction of pancreatic beta cell function are hallmarks of type 1 and type 2 diabetes (15,16). By contrast, non-diabetic obese individuals display increased beta cell mass (15). Although the contribution of NPB/NPBW1 to modulation of energy homeostasis and body weight regulation is well-documented in the literature [reviewed in (4,17)], the role of NPB in controlling pancreatic beta cell functions-insulin expression and secretion as well as beta cell replication-is unknown. Furthermore, NPBW1 signaling in pancreatic beta cells is poorly understood. Thus, in the present study we assessed the effects of NPB on insulin expression and secretion as well as cell proliferation in insulin-producing INS-1E cells [beta cell surrogate (18)] and rat pancreatic islets.

Materials and methods

Reagents. (Des-Br)-Neuropeptide B-29 was purchased from Phoenix Pharmaceuticals (Burlingame, CA, USA). Cell culture media and supplements were from Biowest (Nuaillé, France). 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was from Calbiochem (Merck, Darmstadt, Germany). The BrdU Cell Proliferation kit and Cell Death Detection ELISA PLUS kit were from Roche Diagnostic. Phospho-ERK1/2 (cat. no. 9101S) and ERK1/2 (cat. no. 9102S) antibodies and HRP-linked anti-rabbit antibody (cat. no. 7074S) were from Cell Signaling Technology (Danvers, MA, USA). GAPDH antibody was from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were purchased from Sigma-Aldrich, unless otherwise stated.

Cell culture. Rat insulin-producing INS-1E cells were kindly provided by Professor Pierre Maechler (Médical Universitaire, Genève, Switzerland). Cells were cultured in RPMI-1640 medium supplemented with 10% FBS, 2 mmol/l glutamine, 10 mmol/l HEPES buffer, 1 mmol/l sodium pyruvate, 50 μmol/l beta-mercaptoethanol and 100 kU/l penicillin, 100 mg/l streptomycin.

Isolation of pancreatic islets. Male Wistar rats (body weight 300-350 g) were from Department of Toxicology (Poznań University of Medical Sciences, Poznań, Poland). Animals were sacrificed by decapitation and then the abdominal cavity was opened and the pancreas was filled out by 10 ml of Hanks' buffer containing (in mmol/l): 137 NaCl, 5.37 KCl, 4.17 $NaHCO_3$, 1.26 $CaCl_2$, 0.84 $MgSO_4$, 0.44 KH_2HPO_4, and 0.34 Na_2HPO_4, pH 7.4) supplemented in 1 Wünsch Units/ml of Liberase DL [0.02% (w/v) collagenase; Roche Diagnostic, Germany]. The filled pancreas was immediately cut and placed in a Falcon tube containing 3 ml of Hanks' buffer with Liberase DL. Samples were digested in a water bath (37˚C) for 15 min. This enzymatic process was terminated by the addition of 90 ml 10% FCS in Hanks' buffer. Pancreatic islets were washed several times in Hanks' buffer until the islet preparation was clear. The washing procedure was based on mixing the sample and allowing sedimentation of islets for 3 min. Then, Hanks' buffer with debris was aspirated and sedimented islets were retained. Finally, islets were picked by pipette and transferred to RPMI-1650 medium supplemented with 1% BSA. Then, islets were placed into an incubator for regeneration. After 3 h, the islets were ready for use in all the described experiments.

Reverse transcription-quantitative (RT-q) PCR. Total RNA was isolated using Extrazol (DNA Gdansk, Gdansk, Poland). One microgram of total RNA was reverse transcribed to cDNA using FIREScript RT cDNA Synthesis Mix (Solis BioDyne, Tartu, Estonia). A multiplex RT-qPCR reaction was performed using a QuantStudio 12K Flex (Thermo Fisher Scientific, Waltham, MA, USA). Primers and TaqMan probes were from Life Technologies (Carlsbad, CA, USA). Primers with their Applied Biosystems Assay IDs are as follows: *Npbw1*, Rn01772104_s1; *Npb*, Rn00596187_g1; *Ins1*, Rn02121433_g1; *Ins2*, Rn01774648_g1; *Gck*, Rn00561265_m1; *Glut2*, Rn00563565_m1; *Hnf4α*, Rn04339144_m1; *Mafa*, Rn00845206_s1; *Pdx1*, Rn00755591_m1; *Pgc1α*, Rn00580241_m1; *Hprt1*, Rn01527840_m1. Gene expression was evaluated by the $2^{-\Delta\Delta Cq}$ method; *Hprt1* was used as the endogenous control.

Western blot analysis. The Western blot procedure was performed as previously described (19). Briefly, total protein was isolated using RIPA buffer containing 50 mmol/l Tris-HCl (pH 8.0), 150 mmol/l NaCl, 1.0% NP-40, 0.5% sodium deoxycholate, 0.1% SDS and a protease inhibitor cocktail (Roche Diagnostics). Proteins separated by SDS-PAGE gel electrophoresis were transferred into a nitrocellulose membrane and non-specific binding was blocked using 5% bovine serum albumin in Tris Buffered Saline containing Tween-20 (TBST) for 1 h at room temperature (RT). Thereafter, the membrane was incubated overnight with primary anti-phosphorylated ERK1/2 rabbit polyclonal antibody diluted to 1:1,000 at 4˚C. After washing in TBST, the membrane was incubated with anti-rabbit secondary antibody diluted to 1:5,000 for 1 h at RT. Signals were visualized by enhanced chemiluminescence (ECL kit, Pierce Biotechnology, Rockford, IL, USA). Membranes were further stripped and reprobed for total ERK1/2 and as a loading control GAPDH.

Insulin secretion. INS-1E cells were seeded into 24-well plates ($1.5x10^5$ cells/well) and cultured for 48 h. Following 1 h preincubation in glucose-free Krebs-Ringer-HEPES buffer (KRHB) containing (units: mmo/l) 136 NaCl, 4.7 KCl, 1 $CaCl_2$, 1.2 $MgSO_4$, 1.2 KH_2PO_2, 2 $NaHCO_3$, 10 HEPES (pH 7.4) and 0.1% free fatty acid BSA, cells were washed with KRHB and exposed to 2.8 mmol/l or 16.8 mmol/l glucose in KRHB with or without 100 nmol/l NPB for 60 min. Doses of NPB were chosen based on previous *in vitro* studies (11,20). Next, the medium was collected and centrifuged at 250 x g for 5 min. Insulin content was determined in the supernatants using a High Range Rat Insulin ELISA kit (EIA-3985, DRG Instruments GmbH, Germany, Marburg). Data were normalized to protein concentrations determined by a BCA Protein Assay kit (Thermo Scientific, Waltham, MA, USA).

In the case of pancreatic islets, groups of five rat pancreatic islets of similar size were incubated in KRHB containing 0.1% free fatty acid BSA with or without 100 nmol/l NPB and 2.8 or 16.7 mmol/l glucose for 60 min. Insulin concentration in the incubation medium was measured as described above for INS-1E cells.

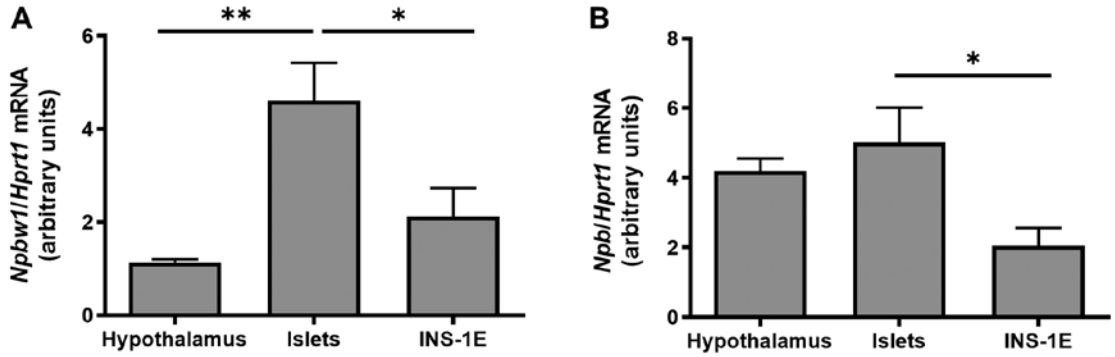

Figure 1. *Npbw1* and *Npb* expression in rat pancreatic islets and INS-1E cells. (A) Reverse transcription-quantitative PCR detection of *Npbw1* in the hypothalamus (positive control), rat pancreatic islets and INS-1E β-cells. (B) *Npb* mRNA expression in the hypothalamus, rat pancreatic islets and INS-1E β-cells. Results are presented as the mean ± standard error of the mean (n=6). *P<0.05 and **P<0.01, as indicated. NPB, neuropeptide B.

Viability and cell proliferation. INS-1E cells were seeded into a 96-well plate ($4x10^4$ cells/well) and cultured for 48 h. After overnight preincubation in serum-free medium, cells were incubated in serum-free medium supplemented with 0.1% free fatty acid BSA and 1, 10 or 100 nmol/l NPB for 24 or 48 h. Cell viability was determined by an MTT assay; cell proliferation was studied using a Cell Proliferation ELISA BrdU kit (Roche Diagnostics) according to the manufacturer's procedure.

Cell death. INS-1E cells ($1.5x10^5$ cells/well) or rat pancreatic islets (8 islets/well) were cultured in 24-well plates and treated with or without NPB (100 nmol/l) for 48 h. Cell death was measured by a Cell Death Detection ELISA PLUS kit (Roche Diagnostics), according to the manufacturer's protocol.

Statistical analysis. Data are presented as the mean ± standard error of the mean. Statistical analysis was performed using GraphPad Prism version 8.0 (GraphPad Software, Inc.), and either a Student's t-test or one-way analysis of variance followed by the Bonferroni post hoc test. Each experiment was repeated independently at least two times. P<0.05 was considered to indicate a statistically significant difference.

Results

Npbw1 and Npb mRNA are expressed in INS-1E cells and rat pancreatic islets. *Npbw1* and *Npb* mRNA were expressed in the hypothalamus (positive control) (4), INS-1E cells and isolated rat pancreatic islets. The highest level of *Npbw1* mRNA expression was observed in rat pancreatic islets (Fig. 1A), while *Npb* mRNA expression levels in the hypothalamus and pancreatic islets were comparable (Fig. 1B). Expression of Npb mRNA in INS-1E cells was lower than in pancreatic islets (Fig. 1B, P<0.05).

NPB stimulates insulin expression and secretion in INS-1E cells. NPB at 100 nmol/l increased *Ins1* mRNA expression (in INS-1E cells after 24 h (Fig. 2B, P<0.05). In contrast, NPB at all tested doses (1, 10 and 100 nmol/l) failed to induce *Ins2* mRNA expression in INS-1E cells after 24 h (Fig. 2B). Furthermore, all NPB doses failed to affect *Ins1* and *Ins2* mRNA expression assessed in cells incubated for 3 or 48 h (Fig. 2A and C).

In addition, cells treated with 100 nmol/l NPB had increased expression of *Mafa* and *Glut2* mRNA (Fig. 2D, P<0.05). In contrast, NPB had no effects on mRNA levels of all other tested genes (Fig. 2D). Since 100 nmol/l NPB was the most efficient dose at increasing insulin mRNA expression, we evaluated the effect of this NPB dose on insulin exocytosis in INS-1E cells. NPB increased insulin release at 2.8 and 16.7 mmol/l glucose (Fig. 2E, P<0.01 and P<0.05). Overall, these data showed that NPB enhanced insulin mRNA expression and secretion in INS-1E cells.

NPB stimulates Ins1 mRNA expression via ERK1/2-dependent mechanism. ERK1/2 modulates insulin mRNA expression in pancreatic beta cells (21). Therefore, we studied the effects of NPB on ERK1/2 phosphorylation. NPB (100 nmol/l) stimulated ERK1/2 phosphorylation in INS-1E cells after 5 min (Fig. 3A, P<0.05).

To study whether ERK1/2 mediates the effects of NPB on *Ins1* mRNA levels, we utilized MEK1/2-dependent ERK1/2 phosphorylation blocker U0126 (22). In the presence of U0126 (10 μmol/l), NPB failed to increase *Ins1* mRNA expression (Fig. 3B). These results show that NPB stimulates *Ins1* mRNA expression via ERK1/2.

NPB fails to modulate INS-1E growth and viability of INS-1E cells. As shown in Fig. 4, NPB at all tested doses (1, 10 and 100 nmol/l) failed to influence INS-1E cell viability (Fig. 4A and C) or proliferation (Fig. 4B and D) after 24 or 48 h. Furthermore, NPB (100 nmol/l) had no effects on INS-1E cell death assessed after 48 h (Fig. 4E). These results show that NPB is not involved in controlling INS-1E cell proliferation and viability.

NPB stimulates insulin secretion but not expression in isolated pancreatic islets. To confirm our findings, we studied the effects of NPB on *Ins1*, *Ins2* mRNA expression, insulin secretion and cell death in isolated pancreatic islets. We found that NPB (100 nmol/l) had no effects on insulin mRNA expression in isolated pancreatic islets after 24 h (Fig. 5A). In contrast, NPB (100 nmol/l) enhanced insulin secretion from pancreatic islets at 2.8 and 16.7 mmol/l glucose (Fig. 5B, P<0.01 and P<0.05). On the other hand, 100 nmol/l NPB did not affect cell death in isolated pancreatic islets (Fig. 5C).

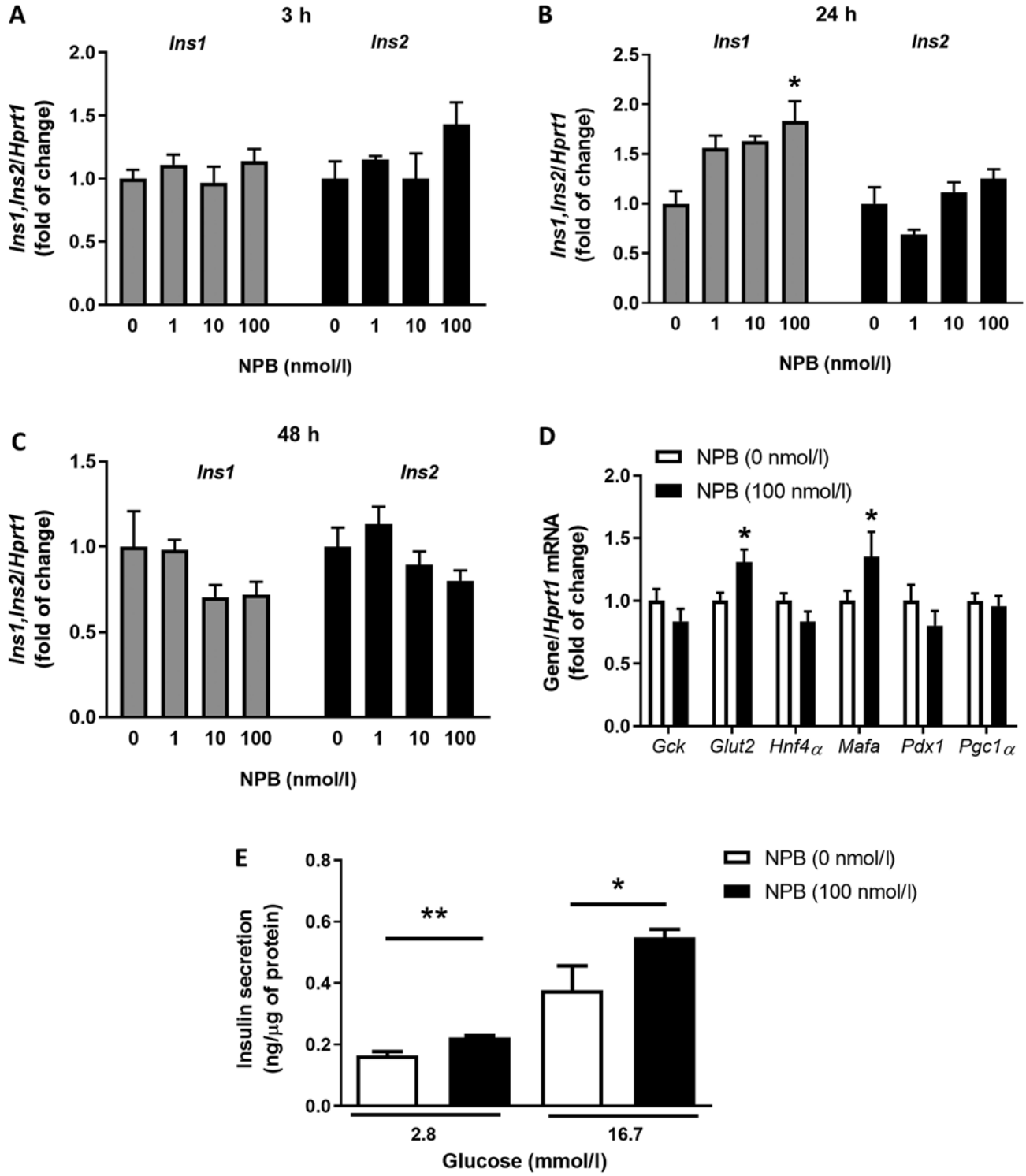

Figure 2. Effects of NPB on insulin expression and secretion in INS-1E cells. The expression of *Ins1* and *Ins2* in cells exposed to NPB (1, 10 and 100 nmol/l) for (A) 3, (B) 24 and (C) 72 h. (D) Expression of *Gck*, *Glut2*, *Hnf4α*, *Mafa*, *Pdx1* and *Pgc1α* in INS-1E cells exposed to 100 nmol/l NPB for 24 h. *P<0.05 vs. 0 nmol/l NPB. (E) Insulin secretion evaluated in cells treated with or without 100 nmol/l NPB in the presence of 2.8 or 16.7 mmol/l glucose for 60 min. Results are presented as the mean ± standard error of the mean (n=5-6). *P<0.05 and **P<0.01, as indicated. NPB, neuropeptide B.

Discussion

In the present study, we report that NPB modulates insulin secretion and expression in INS-1E cells. Furthermore, we demonstrate that NPB stimulates insulin secretion in isolated rat pancreatic islets without affecting insulin mRNA expression and beta cell death.

First of all, we found that *Npb* and its receptor *Npbw1* mRNA are expressed in INS-1E cells and isolated rat pancreatic islets. The presence of NPBW1 and its ligand in insulin-producing cells is in line with previous data demonstrating that NPB and *Npbw1* mRNA are expressed in rat pancreatic islets (8). The presence of NPB in pancreatic islets suggests that this peptide may modulate endocrine cells

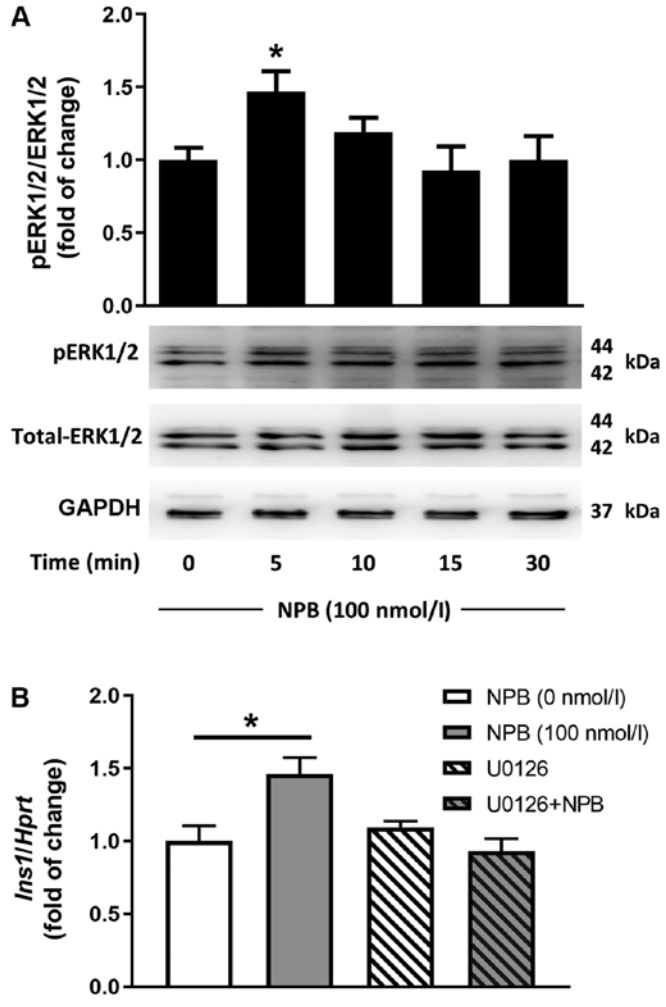

Figure 3. Effects of NPB on ERK1/2 phosphorylation in INS-1E cells. (A) ERK1/2 phosphorylation in INS-1E exposed to 100 nmol/l NPB for the indicated time points. *P<0.05 vs. 0 min. (B) Expression of *Ins1* mRNA in cells treated with or without NPB (100 nmol/l) alone or in the presence of U0126 (10 μmol/l) for 24 h. Results are presented as the mean ± standard error of the mean (n=5-6). *P<0.05, as indicated. NPB, neuropeptide B; p-, phosphorylated.

functions via a paracrine mechanism. However, since our data are restricted to mRNA expression alone, further studies are needed to confirm the presence of NPB on a protein level. Nevertheless, when discussing the role of the NPB/NPW system in pancreatic islets it is worth noticing that there is evidence indicating that another ligand of NPBW1 receptor NPW peptide has been detected with beta cells in rat pancreatic islet (23).

Expression and secretion of insulin from pancreatic beta cells are modulated by a variety of nutritional factors, among which glucose plays the essential role (24). However, there is emerging evidence that numerous appetite-controlling peptides significantly contribute to these processes as well (25-27). Therefore, we studied the influence of NPB on insulin mRNA expression. We found that NPB increased *Ins1* but not *Ins2* mRNA expression in INS-1E cells exposed to NPB for 24 h. Notably, these effects were not detected in cells exposed to NPB for 3 or 48 h, which clearly indicates that the effects of NPB are strictly time-dependent. It cannot be excluded that the lack of an effect on *Ins1* mRNA expression in long-time incubations with NPB was due to the receptor desensitization which can be caused by prolonged exposition of GPCR to their ligands (28).

To explore the mechanism by which NPB increases *Ins1* mRNA expression we assessed its effect on the expression of genes involved in insulin mRNA expression and beta cell metabolism: *Gck*, *Glu2*, *Hnf4α*, *Mafa*, *Pdx1*, *Pgc1α* (14,24,29,30). We found that NPB enhanced *Mafa* and *Glut2* mRNA expression only in INS-1E cell. Previous data showed that in pancreatic beta cells the transcription factor *Mafa* stimulates insulin mRNA expression in a glucose-dependent manner (31). Since NPB also stimulated the expression of the main glucose transporter *Glut2* in rodent beta cells (32), the intracellular glucose content may increase which, in turn, may lead to stimulation of *Mafa*, with concomitant upregulation of insulin mRNA expression. Previous data have demonstrated that binding of *Mafa* to the glucose-responsive A2C1 element of the insulin gene promoter depends on ERK1/2 activation (33). Intracellular cascades downstream of NPBW1 are poorly characterized. However, it has been found that NPB stimulates adrenocortical carcinoma-derived NCI-H295 cells growth via ERK1/2-dependent mechanism (34). Thus, we examined the effects of NPB on ERK1/2 phosphorylation. Our data show that NPB stimulates ERK1/2 phosphorylation in INS-1E cells. Furthermore, we found that the MEK1/2-dependent ERK1/2 phosphorylation blocker U0126 (22) completely blunted the effects of NPB on *Ins1* mRNA expression. Overall, these results suggest that ERK1/2 activation is required to induce *Ins1* mRNA expression in response to NPB treatment.

An open question is why NPB stimulates *Ins1* mRNA expression alone. Notably, there is evidence suggesting the independent regulation of *Ins1* and *Ins2* mRNA expression (35). For example, a mouse study showed that *Ins1* but not *Ins2* mRNA expression is altered by glucose (36). Furthermore, different transcriptional regulation of *Ins1* and *Ins2* mRNA expression was also reported. For example, mice lacking the neuroD transcription factor had reduced *Ins1* but not *Ins2* mRNA expression (37). Moreover, it was shown that Mafa knockout (KO) mice had suppressed *Ins1* but not *Ins2* mRNA expression comparing with wild type animals (38). Thus, NPB may stimulate expression and/or activity of transcription factors (e.g., Mafa) which are predominantly involved in controlling *Ins1* but not *Ins2* mRNA expression.

In addition, our study demonstrates that NPB stimulates insulin secretion from INS-1E cells. These effects were observed at low (2.8 mmol/l) as well as at high (16.7 mol/l) glucose concentrations. These results are comparable with previous studies indicating that activation of the NPBW1 receptor leads to increased insulin secretion in rat islets (23). Dezaki *et al* (23) reported that NPW stimulates insulin release from pancreatic islets; however, this effect was detected at 8.3 mM glucose only and not at 2.8 mM. In this respect, NPB has a higher potency at activating NPBW1 receptor as compared to NPW (39). Thus, this may partially explain the ability of NPB to enhance glucose release even at low glucose concentrations.

Our study lacks an exploration of the mechanism by which NPB triggers insulin exocytosis in beta cells. However, studies on NPW have shown that this peptide stimulates intracellular

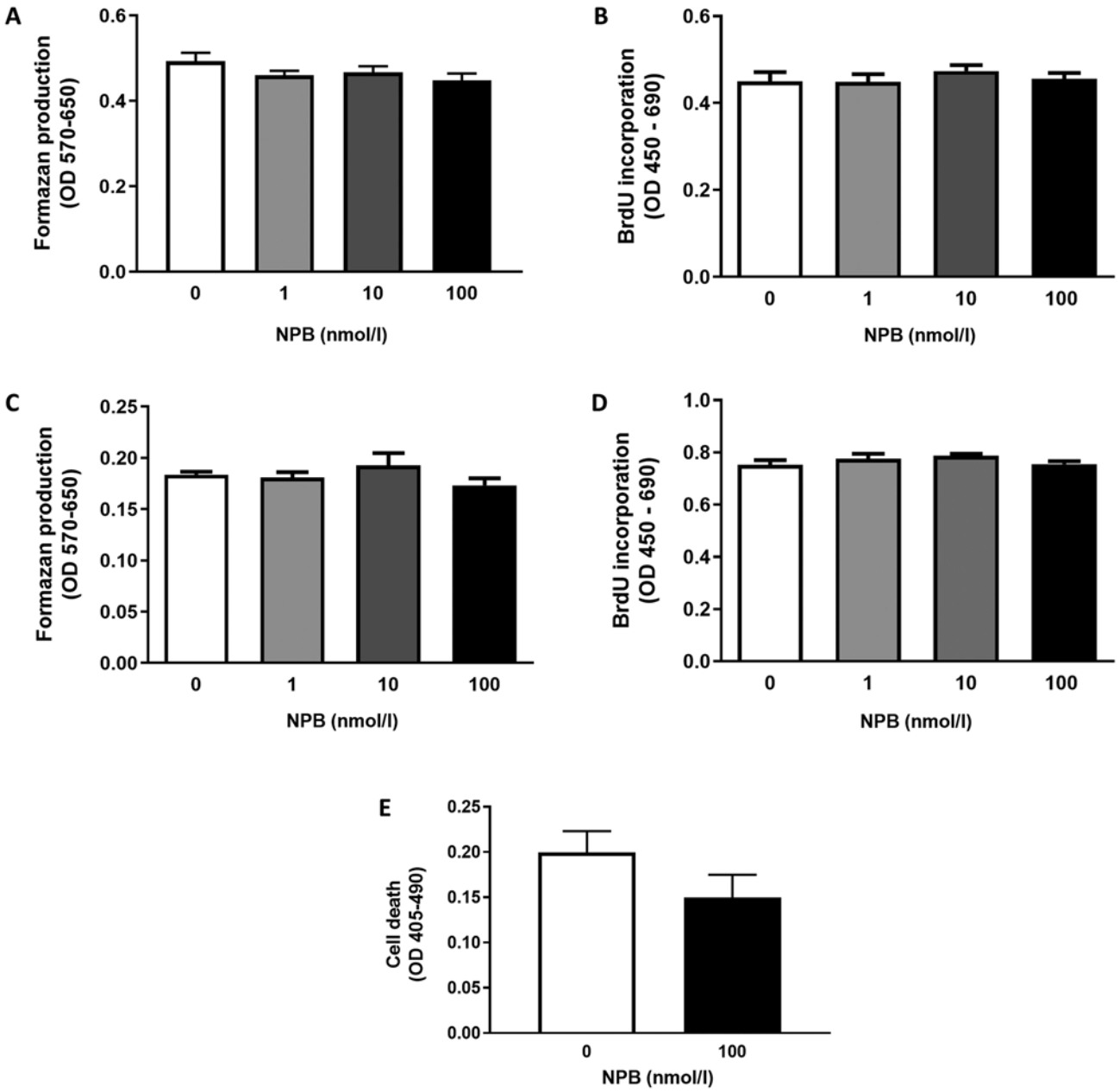

Figure 4. Effects of NPB on the viability, proliferation and cell death of INS-1E cells. Cell viability and proliferation measured by formazan production and BrdU incorporation, respectively, in cells exposed to NPB (1, 10 or 100 nmol/l) for (A and B) 24 or (C and D) 48 h. (E) Cell death was evaluated after 48 h incubation with 100 nmol/l NPB. Results are presented as the mean ± standard error of the mean (n=6-8). NPB, neuropeptide B; OD, optical density; BrdU, 5-bromo-2'-deoxyuridine.

calcium influx in rat beta cells via voltage-gated L-type channels (23). Since both NPW and NPB interact with the same type of receptor (NPBW1) (39), this strongly suggests that NPB may increase insulin secretion by activating the same type of calcium channels.

Type 1 and type 2 diabetes are characterized by a loss of beta cells (40). Identification of new molecular targets able to protect beta cells from death and promote their replication is of clinical importance (41). Therefore, we assessed the effects of NPB on INS-1E cell growth and death. However, we found that NPB did not influence INS-1E cell growth, viability or death. The role of NPB receptor signaling in controlling mitogenesis and cell death is poorly characterized so far. NPB suppresses proliferation of rat calvaria osteoblast-like cells *in vitro* (42). On the other hand, NPB potentiates growth of rat adrenocortical cells (43). Overall, these results suggest that the effects of NPB on cell proliferation are cell-specific. Nevertheless, it should be kept in mind that in our study we used INS-1E insulinoma cells; therefore, the potential influence of NPB on primary beta cell replication remains to be studied in the future.

Finally, to study the relevance of our findings in more physiological settings, we tested whether NPB is involved in insulin expression, secretion and cell death in isolated rat pancreatic islets. We found that NPB increased insulin secretion without affecting insulin mRNA expression or cell death. Therefore, NPB may be a physiological modulator of insulin exocytosis alone. However, it must be noticed that our study is

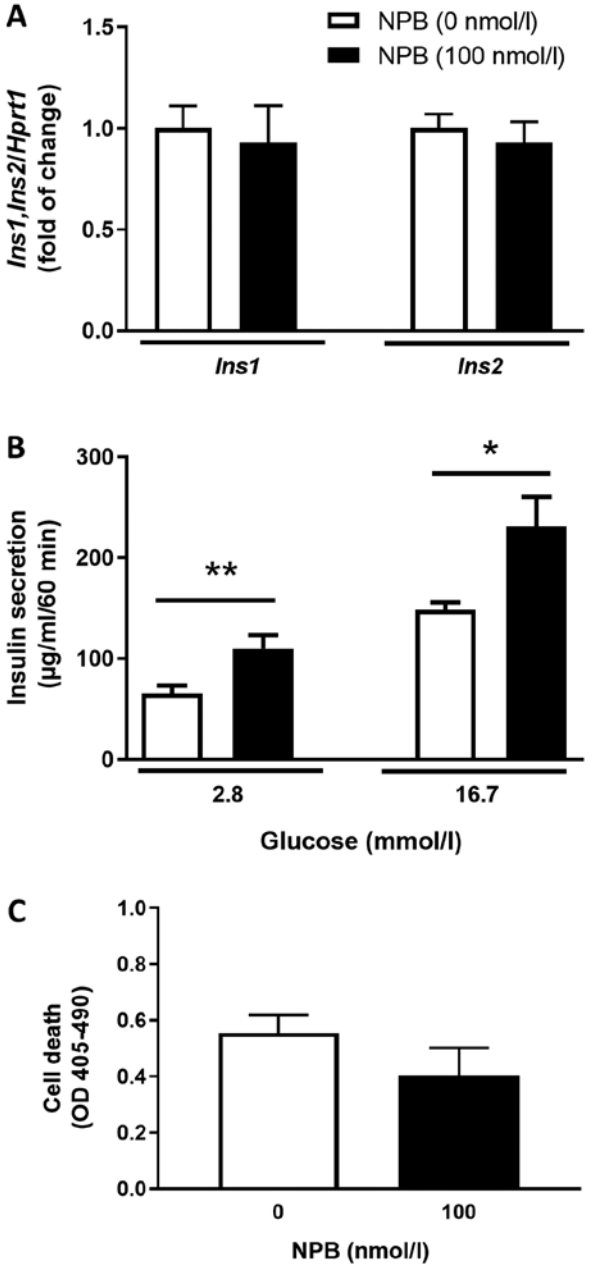

Figure 5. Effects of NPB on insulin mRNA expression and secretion, and cell death in rat pancreatic islets. (A) mRNA expression of *Ins1* and *Ins2* in rat pancreatic islets incubated with or without 100 nmol/l NPB for 24 h. (B) Insulin secretion measured in rat pancreatic islets exposed to 100 nmol/l NPB in the presence of 2.8 or 16.7 mmol/l glucose for 60 min. (C) Cell death evaluated in rat islets treated with or without 100 nmol/l NPB for 48 h. Results are presented as the mean ± standard error of the mean (n=6-8). *P<0.05 and **P<0.01, as indicated. NPB, neuropeptide B; OD, optical density.

limited to static incubation of pancreatic islets. Furthermore, pancreatic islets release other endocrine factors in addition to insulin (44), which in turn may affect insulin mRNA expression. For example, it has been shown that somatostatin which is produced in pancreatic delta cells is able to suppress transcription of the insulin gene (45). In addition, established beta cell lines display different ion channel expressions, glucose sensitivity and numerous aspects of cellular physiology that may differ from those of native beta cells (46,47). Therefore, our results derived from beta cell lines need to be interpreted cautiously. Experiments utilizing purified primary beta cells and/or *in vivo* experiments should answer the questions about the role of NPB in primary beta cell physiology.

In conclusion, we found that NPB stimulates insulin mRNA expression via an ERK1/2-dependent mechanism. Furthermore, we demonstrated that NPB stimulates insulin secretion in INS-1E cells and isolated rat pancreatic islets.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

MB, MSa and MSk designed the study and wrote the manuscript. MB, MSa, TW, MJ and MSk performed the experiments. MZS and KWN interpreted the data, and revised the manuscript. All authors read and approved the final submitted manuscript.

Ethics approval and consent to participate

According to the Act on the protection of Animals used for Scientific or Educational Purposes in Poland adopted on 15th January 2015 and according to earlier regulations, experiments focused on the analysis of tissues obtained after the death of animals that were not treated with any experimental procedure do not require permission of the Local Ethical Commission for investigation on Animals. Therefore, the requirement for ethical approval in the present study was waived. All applicable international, national and/or institutional guidelines for the care and use of animals were followed.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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