Neuropeptides 44 (2010) 485–489

Contents lists available at ScienceDirect

Neuropeptides

journal homepage: www.elsevier.com/locate/npep

Plasma neuropeptide Y levels differ in distinct diabetic conditions ☆

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ARTICLE INFO

Article history:
Received 19 January 2010
Accepted 17 August 2010
Available online 15 September 2010

Keywords:
Neuropeptide Y (NPY)
Diabetes mellitus type 2 (DM2)
Diabetic polyneuropathy (PNP)
Gestational diabetes mellitus (GDM)
Radioimmunoassay

ABSTRACT

Neuropeptide Y (NPY) is an important hormone in appetite regulation. Although the contribution of NPY to metabolic disease has been previously demonstrated, there are only a few reports addressing NPY plasma levels under distinct diabetic conditions. In this study we evaluated NPY plasma levels in diabetes mellitus type 2 (DM2) patients with ($n = 34$) and without ($n = 34$) diabetic polyneuropathy (PNP) and compared these with age and gender matched healthy controls ($n = 34$). We also analyzed NPY plasma levels in gestational diabetes mellitus (GDM) patients with age and pregnancy-week matched controls with normal glucose tolerance (NGT). NPY concentration was determined using a commercially available radioimmunoassay kit. In addition, metabolic parameters of DM2 and GDM patients were recorded. One-way ANOVA tests with appropriate post hoc corrections showed elevated levels of NPY in DM2 patients with and without PNP when compared with those of healthy controls (122.32 ± 40.86 and 117.33 ± 29.92 vs. 84.65 ± 52.17 pmol/L; $p < 0.001$, $p < 0.005$, respectively). No significant difference was observed between diabetic patients with and without PNP. The NPY levels were similar in the GDM group and in pregnant women with NGT (74.87 ± 14.36 vs. 84.82 ± 51.13 pmol/L, respectively). Notably, the NPY concentration correlated positively with insulin levels in DM2 patients ($R = 0.35$, $p < 0.01$). Our data suggest a potential involvement of circulating NPY in DM2 pathology.

1. Introduction

Functional characterization of peptide hormones in physiological as well as pathological processes is important for clinical treatment strategies. Neuropeptide Y (NPY), which belongs to the pancreatic polypeptide family, was initially isolated from porcine brain and suggested to be an orexigenic protein (Tatemoto, 1982; Tatemoto et al., 1982). NPY is one of the most frequently investigated hormones in appetite regulation and is found in diverse tissues with a predominant localization in the central nervous system (Bai et al., 1985). It is synthesized and stored in the arcuate nucleus of the hypothalamus, where it acts as a neurotransmitter (Allen et al., 1983; Gray and Morley, 1986).

NPY in the hypothalamus has been shown to be physiologically important in regulating food intake and energy consumption. It was suggested to be the primary responder to both short-term and long-term fasting conditions (Bi, 2007). Chronic administration of NPY resulted in sustained hyperphagia and ultimately obesity in mouse models (Beck et al., 1992; Stanley et al., 1986). Hypothalamic NPY is also increased in genetically induced obese diabetic (db/db) mice (de Luca et al., 2005). However, whether NPY over-expression is causative or associated with obesity in this model remains to be elucidated. In diabetic rats, NPY expression in hypothalamic regions was enhanced, whereas after insulin injection its expression was decreased (Sipols et al., 1995). NPY was also shown to function in pancreatic beta cell proliferation (Cho and Kim, 2004) and was over-represented in the plasma of non-insulin dependent diabetes mellitus in obese women (Milewicz et al., 2000).

Outside the central nervous system, NPY is expressed in sympathetic neurons. From these neurons, NPY is released into the endocardial endothelial cells, the gut and the spleen (Cox, 2007; Ericsson et al., 1987; Jacques et al., 2006). Additionally, elevated NPY levels were found under stress conditions (Bernet et al., 1998). Furthermore, expression of NPY within adipocytes may contribute to adiposity (Kos et al., 2007) and its metabolic consequences.

☆ This work was supported by the Jubilaeumsfonds of Austrian National Bank, Project Number: 13402.

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doi:10.1016/j.npep.2010.08.001

Thus, NPY might be involved in the pathogenesis of metabolic disorders including obesity, anorexia nervosa and DM2 (Baranowska et al., 2003). However, there exists little information regarding the plasma levels of NPY in diabetic patients with and without diabetic complications. To address this, we evaluated NPY plasma levels of DM2 patients with and without diabetic neuropathy and compared them to age and gender matched healthy controls. In addition, we investigated whether gestational diabetes mellitus, a distinct type of diabetes, and DM2 patients share similarities in plasma NPY concentration. For this purpose, a pregnant group with normal glucose tolerance was also included. Furthermore, NPY concentration was correlated with patients' characteristics and metabolic status in DM2 and GDM groups.

2. Methods

2.1. Study population

In the current study we analyzed plasma NPY concentration in patients suffering from diabetes mellitus type 2 (DM2) with ($n = 34$) and without polyneuropathy (PNP) ($n = 34$) and age and gender matched healthy controls ($n = 34$) as well as subjects with gestational diabetes mellitus (GDM) ($n = 30$) and age and pregnancy-week matched control subjects ($n = 30$) with normal glucose tolerance. The exclusion criteria for the diabetic patients were: advanced renal failure (Glomerular filtration rate <15 ml/min/1.73 m2), advanced liver failure (according to Child-Pugh Classification for Severity of Liver Disease: bilirubin >3 mg/dL, albumin <2.8 g/L, prothrombin time >6 s, hepatic encephalopathy and severe ascites), undergoing chemotherapy, hyperthyroidism, Cushing Syndrome and hyperparathyroidism. In order to select diabetic neuropathy patients, all diabetic patients were assessed with foot tests including distal sensory and motor nerve examination with microfilament tests and examinations of foot reflexes. To confirm the diagnosis of diabetic neuropathy, nerve conduction velocity of the upper and lower extremities was evaluated.

The patients suffering from DM2 and the subjects with GDM were included from the out-patients clinic of the Department of Endocrinology and Metabolism, Medical University of Vienna. Blood collection was performed in the morning at 8.00 a.m. after overnight fasting. Written informed consent was received from each patient prior to vein puncture. The study was approved by the local Ethics Committee.

2.2. Sample collection

Blood collection was performed using a 7 ml vacutainer EDTA-tube. After cooling immediately on ice, the plasma was separated from cells by centrifugation at 3200 rpm for 10 min at 4 °C. The plasma was distributed into aliquots and frozen at −80 °C for further use. The exact time points of blood sampling and freezing were documented. For the DM2 and GDM patients, metabolic parameters including insulin, HbA1c, C-peptide and blood glucose were measured as a routine procedure.

2.3. NPY radioimmunoassay

Plasma NPY concentration was analyzed using a commercially available NPY radioimmunoassay kit (Euro-Diagnostica, Malmö, Sweden). The assay was performed according to the user's manual. Briefly, the standard was prepared with provided standard solutions. 100 μl of either standard or plasma sample was pipetted in the respective tubes. Zero standard and total radioactivity tubes were also prepared. This was followed by addition of 100 μl of anti-NPY monoclonal antibody. Tubes were thoroughly mixed and incubated for 24 h at 4 °C. After this period, 100 μl of ^{125}I-NPY was added to all tubes and mixed by vortexing. After incubation period of 24 h at 4 °C, 100 μl of double antibody solid phase was pipetted to all tubes and incubated for 60 min at 4 °C. The tubes were then centrifuged for 15 min at 4 °C at 1800 rpm. Supernatants were decanted immediately after centrifugation. The radioactivity of the individual precipitates was evaluated by a gamma counter.

2.4. Statistical analysis

The NPY levels were tested for normal distribution using the Kolmogorov–Smirnov test and for homogeneity using Levene's test. This did not violate criteria for parametric tests. Differences between group means were analyzed using one-way ANOVA followed by post hoc comparisons using Tukey's test. In order to adjust the influence of covariates on the NPY concentration, a univariate general linear model selecting the age and BMI for covariates was generated. NPY concentrations and metabolic parameters of the patients were correlated using Pearson's correlations coefficient. P values <0.05 were considered to be significant. All data were presented as mean ± SD. All statistical calculations were performed with SPSS, Version 18 (Chicago, Illinois). Graphs were created using Prism Graph Pad (La Jolla, CA).

3. Results

3.1. NPY in DM2

We determined concentrations of NPY in plasma derived from DM2 patients with ($n = 34$) and without PNP ($n = 34$) and age and gender matched controls ($n = 34$). Multiple group comparisons showed a significant difference between the groups analysed (one-way ANOVA, $F\ (2, 99) = 8.08$, $p < 0.001$). The subsequently performed Tukey's group comparisons showed an elevated level of NPY concentration in the DM2 patients with and without PNP when compared with the healthy control group (122.32 ± 40.86 and 117.33 ± 29.92 vs. 84.65 ± 52.17 pmol/L; $p < 0.001$, $p < 0.005$, respectively) (Fig. 1). This remained significant even after adjustment for age and BMI. In contrast the mean NPY concentration did not differ within the group of DM2 patients with and without PNP.

In our patient cohort, metabolic parameters were also recorded. Fasting blood glucose, insulin, C-peptide and HbA1c concentrations were measured as routine procedure and the BMI of the patients was calculated. Patient characteristics and the concentrations of

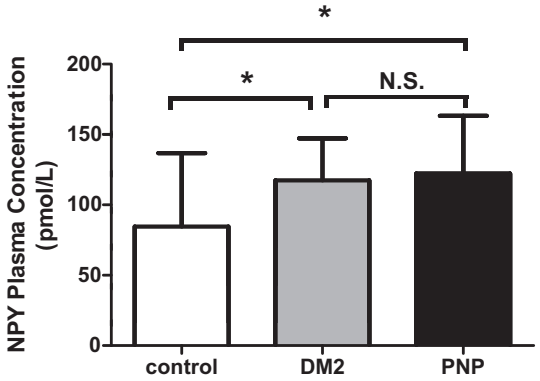

Fig. 1. NPY plasma concentrations of healthy controls and diabetic patients with and without polyneuropathy. Data are presented as mean ± SD. *$p < 0.01$ with ANOVA, corrected by Tukey's test. DM2: diabetes mellitus type 2; PNP: polyneuropathy; N.S.: not significant.