NPY Levels In Type 1 Diabetic Men of Different Duration.

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Abstract

Background: The aim of the present study was to evaluate whether the different duration of type 1 diabetes mellitus influences basal NPY secretion.

Design: The NPY concentrations were measured in sixty-eight men with insulin-dependent diabetes mellitus (IDDM) (duration: group 1 (n.21) <5 years (range 2-4 years); group 2 (n.24) >5 years and <10 years (range: 6-9 years); group 3 (n.29) >10 years (range: 11-14 years)) and in age matched normal control subjects (n. 30).

Results: The NPY levels were significantly lower in group 3 than in group 2 and 1 and in the control group, in group 2 than in group 1 and in the control group and in group 1 than control group.

Conclusion: These results support the view that the duration of diabetes may have a modulatory role in the decreased basal NPY secretion observed in diabetics.

Introduction

The metabolic disturbance of diabetes mellitus (DM) might cause adaptive change in the hypothalamic regulation of metabolic control. Such changes may involve regulatory peptides, which are known to affect food intake and glucoregulation[1-3].

Disturbance of hypothalamic regulatory peptides could be associated with the impairment of hormonal activity in diabetes[4,5]. The identification of local regulatory peptides activity in diabetes may therefore help to clarify hypothalamic control mechanisms. NPY is one of the most widely spread neuropeptides in the rat brain. Large amount of NPY are present in several hypothalamic sites and immunopositive NPY fibers emanating from NPY producing perikarya in the hypothalamic arcuate nucleus and brain stem innerve several hypothalamic regions[6,7]. In addition, NPY plays an important role in the neuroendocrine regulation of eating behaviour[1-3, 8-13].

Disturbances in NPY function has been observed in diabetes mellitus[14,15]. Altered hypothalamic NPY levels may mediate hyperphagia and polydipsia in diabetic rats[16,17] and thus disorders of NPY activity might exert an important influence on metabolic control in diabetes.

Previous studies indicated that different duration of insulin dependent diabetes mellitus (IDDM) play a role in the control of LH and AVP secretion. In fact diminution in LH and AVP secretory patterns appeared to be more evident in patients with time after the onset of IDDM[18-20]. In light of the difference in LH, AVP secretion, we wondered whether a similar difference also concerns NPY secretions. For this purpose, NPY circulating concentrations were measured in diabetic men affected by IDDM of different duration. The results obtained in these groups were compared with those observed in control age-matched normal men.

Subjects and methods

Fifty-eight insulin-dependent diabetic men, 23 to 46 years, participated in this study. The duration of diabetes ranged from 2 to 14 years. Patients were divided into three group of subjects according to the duration of their disease (group 1 (n. 21): <5 years, range 2-4 years; group 2 (n. 24): >5 years and <10 years, range 6-9 years; group 3 (n. 23): >10 years, range 11-14 years). The mean age ± SE was 29.3±1.8 years in subjects of group 1, 31.2±1.4 in subjects of group 2 and 32.6±1.8 in subjects of group 3. Thirty normal men (27 to 37 years of age) with normal body weight and without signs of endocrine diseases or family history of diabetes mellitus participated in this study as controls. All subjects were informed of the purpose of the study which was performed according to the Helsinki II declaration. The Ethical Committee of the University of Parma, rewieved the protocol of the study. None of the diabetic and control subjects were affected by major affective disorders as determined by the Hamilton Depressive Rating Scale. None of the diabetic patients received any drug, except insulin because of diabetes mellitus, for at least 20 days before the experimental days. From the onset of their illness, the patients had been treated with insulin and at the time of this study they were hospitalized for adjustment of insulin therapy. Venous blood samples were drawn

at 08.00 after a 12 h overnight fast after optimization of insulin administration, which was similar in both groups (dose of short acting and intermediate duration monocomponent insulin given together twice daily 31.2 ± 2.0 IU/24h (mean ± SE) (group 1), 30.4 ± 1.9 IU/24 h (group 2), 32.0 ± 1.9 IU/24h group 3. Insulin therapy did not change because the subjects had achieved a good control of their metabolic status [average blood glucose: 7.9 ± 0.8 nmol/l (mean ± SE) (group 1), 7.8 ± 0.6 (group 2), 7.7 ± 0.6 group 3; percent of glycosilated haemoglobin (HbA1c): 7.0 ± 0.8 (mean ± SE) (group 1), 7.3 ± 0.7 (group 2), 7.4 ± 0.6 group 3]. No patient had clinical or laboratory evidence of ketosis or of associated endocrine or other intercurrent diseases. No signs of autonomic or somatic nerve dysfunction or vascular disease were present in any patients. In all of them creatinine clearance was in the normal range of values (96.4 ± 4.3 ml/min (mean ± SE) (group 1), 96.2 ± 3.9 (group 2) 97.6 ± 21.0 group 3; microalbuminuria was absent in all subjects. The mean (± SE) blood pressure was 96.5 ± 1.1 mmHg (group 1), 98.1 ± 1.4 mmHg (group 2), 97.8 ± 1.3 (group 3), 97.1 ± 1.4 mmHg (controls). All patients underwent ophthalmologic examination to detect the presence of diabetic retinopathy.

Diagnostic criteria. After papillary dilatation patients underwent ophthalmoscopic examination performed by the same experienced opthalmologist. The finding of at least three microaneurysms was considered a sign of retinopathy. Patients with retinopathy were excluded from the study. As control indices of the metabolic status of these patients, blood glucose levels were measured at 7.30 AM, 11.00 AM and 05.00 PM on the day preceding this experimental study. For each patient, a mean value of blood glucose was obtained by averaging these three determinations. On the experimental day, blood samples were taken for evaluation of glycaemia and HbA1c.

Blood glucose concentrations were measured with a glucose oxidase-peroxidase procedure, using an IL 918 autoanalyzer (Instrumentation Laboratory, Milan, Italy). HbA1c was measured with reagents obtained from Bio-Rad Laboratories (CA, USA). In our laboratory the normal range of values for HbA1c is 4.1-6.5%. Plasma NPY levels were measured by RIA utilizing commercial kits. The intra-assay and inter-assay coefficients of variation were: 3.6% and 11.0%, respectively.

The sensitivity of the method was 6 pmol/l. Results were analyzed statistically with non-parametric Mann-Whitney U test, and linear correlation coefficient of variation as appropriate. All data are reported as mean ± SE.

Results

Clinical and biochemical characteristics of diabetic patients and normal controls are shown in Table 1.

Plasma NPY levels were significantly lower in group 2 than in group 1 ($P<0.01$) and normal controls ($P<0.01$). In group 1 NPY concentrations were lower than in normal controls ($P<0.01$) (Fig. 1). When data obtained in patients of group 1, group 2 and group 3 were combined, significant negative correlations were found between duration of diabetes mellitus and NPY concentrations ($r=0.720$; $P<0.01$).

Discussion

Our data suggest that changes in plasma NPY levels in diabetics are influenced by the duration of the diabetic condition. On the other hand, our patients were in similar metabolic status at the moment of the study; and thus the alteration in NPY concentrations cannot be attributed to the intercurrent factors such as decreased body weight, poor nutritional conditions or chronic organic disorders. More likely the abnormal NPY levels depended on the diabetic conditions per se. A limitation of the present study is the measurement of a single fasting hormone concentration. However, fasting NPY concentrations is a representative sample of baseline conditions, without the influences of pre-prandial or post-prandial factors because NPY concentrations may be influenced by food intake. In fact, the evaluation of fasting hormone level is usually a reference point to compare two or more populations under different conditions. Because of these reasons, we decided to evaluate NPY levels in fasting conditions.

The source of circulating NPY concentration remains unclear. NPY is mainly reported to be present in the hypothalamic sites including paraventricular and arcuate nucleus[21]. The blood brain barrier (BBB) inhibits the release of peptides into the circulation from various structures in the brain; however the arcuate nucleus is known to be a region where the BBB is absent[22]. Therefore, the secretion of NPY from this structure into the blood stream is possible.

NPY as been also found in the autonomic sympathetic nerve. A recent study reported a mouse model which over-expression NPY exclusively in the noradrenergic neurons to evaluate its systemic actions when expressed outside of the hypothalamus and the BBB[23]. In fact, although they described an increase of NPY within sympathetic neurons, its blood concentrations was not altered in rats. Therefore it is unlikely that autonomic sympathetic neurons are a source of circulating NPY.

The progressive decline of NPY levels with long-term IDDM might be explained by diabetes-related alteration of the NPY cell but also it is possible that an unstable glycemic control lasting for a long time produced neuroendocrine damages due to relative insulin deficiency; these alterations were not reversed by 3 days of continuous insulin infusion before the experiments. Previous reports showed decreased concentrations of plasma NPY in subjects with type 1 diabetes mellitus[24]. In contrast, it has been showed an elevation of NPY in type 2 diabetes mellitus patients[25,26]. In diabetics, this suggests that differences in the metabolic status of diabetics might affect the circulating concentrations of NPY. As to how relative insulin deficiency may produce NPY alterations[14] and the site of this action are still unknown.

A question rising by our data concerns the consequences of the decreased NPY secretion. Injection of NPY is known to stimulate appetite[27,28]. Therefore the change in ingestive behaviour in diabetes mellitus may be attributed to an altered release of NPY.

The activity of NPYergic neurons in the hypothalamic centers involved in these functions may be responsible for the multitude of effects on reproductive, feeding and sexual behaviour in diabetic subjects[27,28]. Our data suggest that NPYergic activity is strongly related with time after the onset of IDDM. Further studies are needed to substantiate these hypotheses.

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Legend to the Fig. 1

Basal NPY levels (mean ± SE) in normal controls and in Group 1 and in Group 2 of patients with diabetes mellitus.

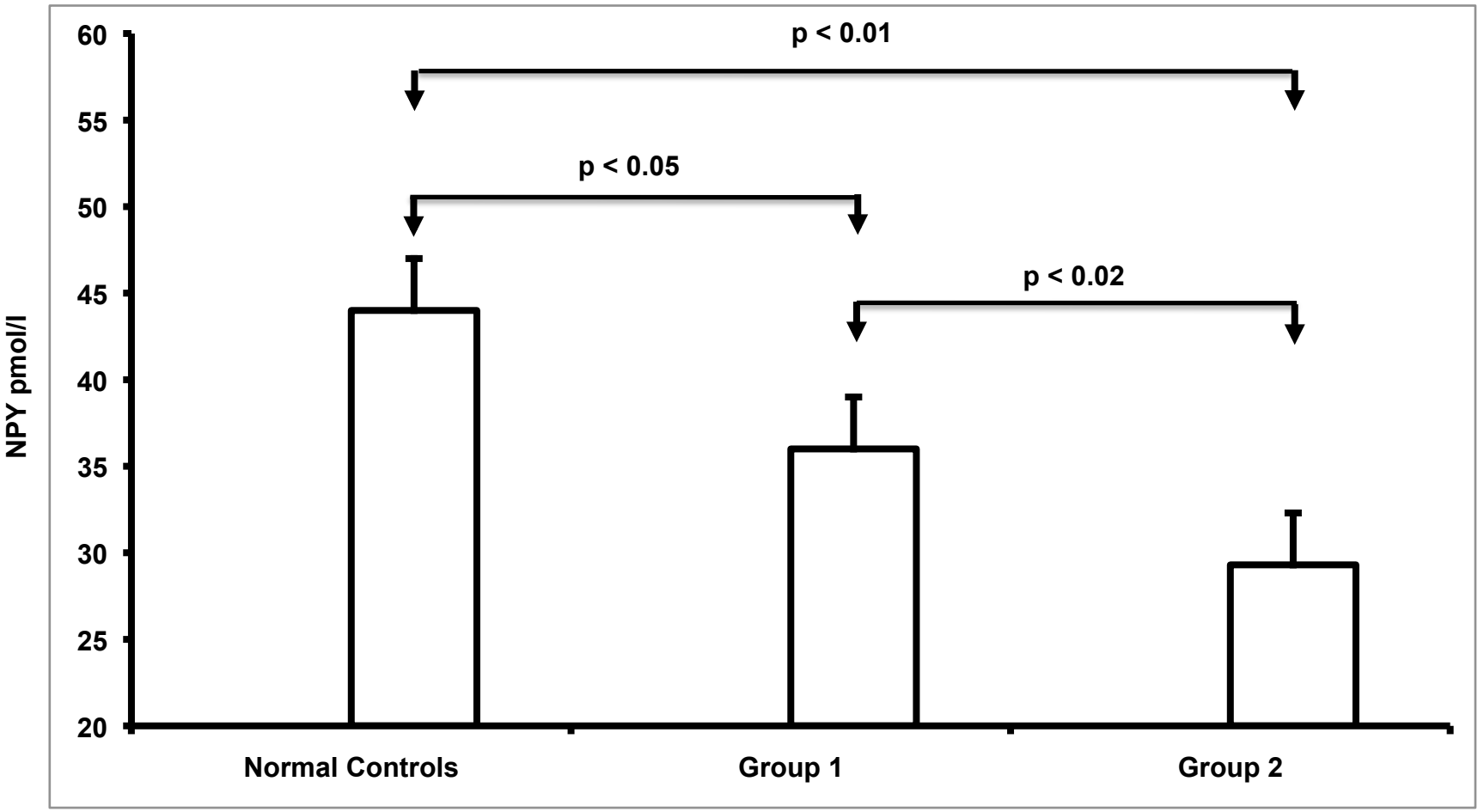

Table 1 Clinical and biochemical data of the diabetic men (groups 2 and 3) and normal controls

	Age (years)	Weight (kg)	BMI	Duration of IDDM	Blood glucose ($mmol/L^{-1}$)	HbA1c (%)
Group 1 (n= 21)	29.3±1.8	65.4±1.4	22.0±0.5	<5 (2-4 years)	7.9±0.8	7.0±0.8
Group 2 (n= 23)	31.2±1.4	64.8±1.7	22.6±0.6	>5 and <10 (6-9 years)	7.8±0.6	7.3±0.7
Control group (n= 30)	32.7±1.6	67.4±1.8	23.4±0.4		4.7±0.2	5.6±0.3

BMI = Body mass index