
Autonomic dysfunction and neuropeptide Y in fibromyalgia

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ABSTRACT

Objective. *Fibromyalgia (FM) is a syndrome associated with widespread pain and various other signs and symptoms. Several of these multisystem features could be explained on the basis of autonomic nervous system (ANS) dysfunction.*

Methods. *The aim of the present study was to evaluate ANS dysfunction in FM based on time-domain heart rate variability (HRV) analysis and serum neuropeptide Y (NPY) levels in 51 patients with FM, 25 patients with systemic sclerosis (SSc), and 15 healthy controls (NHS).*

Results. *Compared with the SSc and NHS groups, the FM group had significantly higher NPY levels, and in the FM subgroup subjected to HRV analysis (25/51 patients, 49%), certain HRV indices were significantly reduced. In this subgroup, NPY was significantly correlated with the SDANN index and the NN50, but neither NPY or HRV parameters showed any significant correlation with clinical aspects of the FM.*

Conclusion. *These findings suggest that autonomic dysfunction and NPY are crucial elements in the pathophysiology of FM. Additional studies are necessary to define the complex roles played by NPY and ANS in modulating pain and immunological functions of different diseases.*

Introduction

Fibromyalgia (FM) is a syndrome characterised by widespread pain associated with a variety of other signs and symptoms, such as fatigue, sleep disorders, paresthesias, headache, anxiety, sicca symptoms, Raynaud's phenomenon, and irritable bowel (1, 2). Several of these multisystem features could be explained on the basis of autonomic nervous system (ANS) dysfunction. The ANS is a complex regulatory system, which preserves homeostasis and plays a major role in the stress response

system. The sympathetic/parasympathetic balance is crucial for normal ANS function. Some lines of evidence suggest that ANS dysfunction is involved in FM. As a matter of fact (3, 4), several parameters of autonomic function are significantly altered in FM patients compared with healthy controls (5-7). Moreover, alterations of the sympathetic nervous system may lead to chronic pain and allodynia. Sympathetic hyperactivity, as well as catecholamines and neuropeptides, are known to activate primary afferent nociceptors (8).

A simple non-invasive method for quantifying the activity of ANS functions is heart rate variability (HRV) analysis (9). It is based on the fact that heart rate is not constant. It oscillates around a mean value as a result of variations in the activity of the ANS, which control the heart rate through the sympathetic and parasympathetic systems. The term HRV refers to the cyclic changes observed over time in the sinus rate (8). Analysis of HRV provides quantitative information on autonomic tone as reflected by the effects of control mechanisms.

Neuropeptide Y (NPY) is a neurotransmitter released mainly by sympathetic neurons, and its expression in the ANS is colocalised with that of norepinephrine. It is considered a good indicator of sympathetic activity, and compared with norepinephrine, it offers the advantages of greater stability and a longer plasma half-life. Studies conducted over the past two decades have delineated complex roles for NPY and its Y1 and Y2 receptors in the modulation of pain. In fact, NPY has been shown to cause pain but also to reduce it (10).

The aim of the present study was to investigate ANS function in patients with FM by analyzing HRV and serum NPY levels.

Patients and methods

HRV and serum NPY levels were assessed in 3 groups of subjects recruited

Competing interests: none declared.

consecutively from the Rheumatology Unit of the I University of Rome Medical Center. The first group consisted of 51 women (mean age 49.6 yrs, range 23–75 yrs) with primary FM diagnosed according to American College of Rheumatology criteria (1). The second included 25 patients (24 women, 1 man; mean age 51.73 yrs, range 20–68 yrs) with systemic sclerosis (SSc) diagnosed according to ACR criteria (11), and the third comprised 15 healthy controls (12 women, 3 men; 48.6 yrs, range 20–66 yrs). Exclusion criteria were the presence of cardiac disease, hypertension, diabetes mellitus, and/or neurological diseases as assessed by history, physical examination, and standard 12-lead ECG. None of the participants were taking vasodilators, antiarrhythmics, or neurological drugs. After providing informed consent to all study procedures, the patients were interviewed, and complete medical histories were recorded. Clinical and rheumatologic examinations were also performed. The assessment of patients with FM included a tender points (TP) count, administration of the Fibromyalgia Impact Questionnaire (FIQ) and Health Assessment Questionnaire (HAQ), and Visual Analogue Scale (VAS) ratings of pain, fatigue, stiffness, anxiety, depression, and disease activity. A 10-ml specimen of venous blood was then drawn from each patient between the hours of 8:00 and 9:00 am. The serum was separated and stored at -20°C until assayed.

Laboratory analysis

Serum levels of NPY were measured with a commercial immunoenzymatic assay kit (Phoenix Pharmaceuticals, Inc.; USA) in accordance with the manufacturer’s instructions. Results were expressed in nanograms per milliliter.

Heart rate variability

Holter monitoring (24-hour ambulatory ECG recording – Rozinn Electronics H4W 3.6F, Glendale, NY) was performed in the first 25 of the 51 patients with FM (mean age 48.9 yrs, range 23–65 yrs), all 25 of those with SSc, and all 15 of the healthy controls. A template recognition algorithm was used

Table I. Serum NPY levels in patients with FM or SSc and in healthy controls.

Group	NPY ng/mL median (range)	p-values
FM n=51	3.9 (0.8 ± 7.1)	p<0.0001 vs NHS p=0.0001 vs SSc
SSc n=25	2.5 (0.9 ± 7.1)	NS vs NHS
NHS n=15	2.5 (0.9 ± 6.1)	

*NHS: normal human sera.

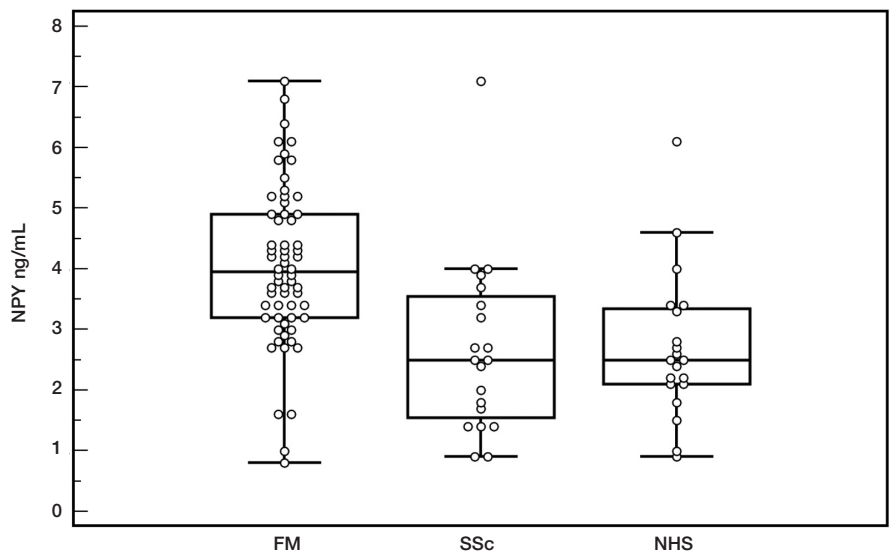


Fig. 1. NPY levels in the FM, SSc, and healthy control groups.

to reject noisy or abnormal complexes. HRV was evaluated in the time domain using appropriate software, computing the time series of all normal-to-normal (NN) QRS intervals throughout the 24-h recording period. We calculated the standard deviation (SD) of all NN intervals (SDNN); the SD of the average NN interval in each 5-min segment of the recording (SDANN); the SD of the average normal R-R interval for each 5-minute segment (SDANN Index); the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD); and the pNN50, i.e. the number of pairs of adjacent NN intervals differing by more than 50 ms in the entire recording (NN50) divided by the total number of NN intervals (12).

The simplest way to perform HRV measurements is time domain analysis. The principal variables – the SDNN, the SDANN, and the SDANN index – reflect overall HRV and provide better prognostic information. The SDANN

and RMSSD estimate the long-term and short-term components of HRV, respectively. The RMSSD and pNN50 are specific markers of parasympathetic control and strongly reflect vagal tone, but the RMSSD is preferred because of its better statistical properties. Moreover, the SDNN and SDANN index are reflections of the circadian rhythm of heart rate (12).

Statistical analysis

The statistical analysis was based on the use of chi-square statistics or Fisher’s exact test (if n<5) for independence and appropriate t-tests. Linear regression was used to assess correlations. When necessary, appropriate nonparametric tests were employed (Spearman correlation test, Kruskal-Wallis ANOVA by Ranks, Mann-Whitney U-test). Data are presented as means+SD or medians (interquartile range; IQR). Only two-tailed probabilities were used for testing statistical significance. P-values lower than 0.05 were regarded as statistically

Table II. Time-domain HRV measures in patients with FM or SSc and in healthy controls.

Variable	FM (n=25) median (range)	SSc (n=25) median (range)	NHS (n=15) median (range)	p-values
SDNN (ms)	99.7 (50.8–172.6)	113.1 (63.1–181.6)	133.0 (96.3–225.7)	$p=0.005$ FM vs NHS $p=0.032$ SSc vs NHS
SDANN (ms)	70.4 (36.1–128.3)	207.3 (53.9–174.9)	111.6 (84.2–189.9)	$p=0.0001$ FM vs NHS $p=0.0013$ FM vs SSc
NN50 count	2169.0 (107.0–18,220.0)	2169.0 (107.0–18,220.0)	3987.0 (525.0–21,865.0)	$p=0.03$ FM vs NHS
pNN50 (%)	3.3 (0.1–22.7)	2.9 (0.3–20.0)	4.1 (0.4–37.0)	NS FM vs SSc NS FM vs NHS
SDANN index (ms)	39.0 (19.2–55.7)	44.8 (23.2–51.3)	51.07 (35.9–111.4)	$p=0.01$ FM vs SSc $p=0.001$ FM vs NHS
RMSSD (ms)	27.3 (13.5–152.8)	28.8 (14.8–61.7)	27.4 (15.4–74.1)	NS FM vs SSc NS FM vs NHS

significant. All calculations were carried out with Statistica software (StatSoft Inc., Tulsa, OK, USA).

Results

Serum NPY levels were significantly higher in the FM group than in the SSc patients and healthy controls (Table I, Fig. 1). Analysis of the 25 patients with FM who underwent HRV analysis (Table II) revealed significantly lower values of SDNN, SDANN, and SDANN index compared with those of the SSc group and healthy control group. The NN50 count for the FM patients was also lower than that of the healthy controls. In the FM subgroup subjected to HRV analysis, NPY levels displayed specific correlations with the SDANN index ($p=0.009$) and the NN50 ($p=0.02$), but neither the HRV parameters nor the NPY level was significantly correlated with clinical variables (TP count, FIQ and HAQ scores, VAS scores).

Discussion

HRV analysis is based on the well-known fact that the heart rate is not constant but varies constantly and randomly from beat to beat. This continuous variability is due to the reciprocally antagonistic effects of the sympathetic and parasympathetic system on the sinus node. HRV can be studied in both the time domain (where the basic units are milliseconds) and the frequency domain with spectral analysis (the basic units are Hertz; cycles/sec) (8). Studies of ANS function in FM patients based on both methods have demonstrated

alterations of the sympathetic/parasympathetic balance consisting of sympathetic hyperactivity (5, 13–17). In time-domain analysis, this imbalance, which is characterised by increased sympathetic activity and/or reduced parasympathetic activity, is reflected by reduced HRV parameters (18).

In the present study, time-domain HRV analysis was used to compare ANS function in a group of patients with FM and in two control groups, one composed of healthy subjects, the other of patients with SSc. We chose time-domain analysis because it provides a more faithful reflection of physiological ANS function, whereas frequency-domain analysis entails tilt table testing, which represents an acute stress. Autonomic dysfunction is a well-recognised component of SSc, as demonstrated by a previous study by our group (19). This disease is characterised by parasympathetic dysfunction associated with sympathetic over-activity and depression of the circadian rhythm of heart rate (20–22).

In the present study, the principal HRV measures (SDNN, SDANN, SDANN index, and NN50 count) in the FM group were significantly lower than those of the SSc and healthy control groups, reflecting altered autonomic function characterised by sympathetic hyperactivity.

We also investigated autonomic function as reflected by serum levels of NPY, a 36-amino-acid peptide originally described by Tatemoto *et al.* (23). This highly conserved neurotransmit-

ter is widely distributed throughout the central and peripheral nervous systems (24). It is well known that NPY co-localises with norepinephrine in the sympathetic nervous system, and elevated NPY levels are linked with strong sympathetic activation (25). Serum levels of NPY in our patients with FM were significantly increased over those found in healthy subjects or patients with SSc.

To our knowledge, only one other study has demonstrated significantly elevated NPY levels in FM patients, and as in our patients, these increases displayed no significant correlation with pain (26). This finding suggests that ANS and NPY alterations in FM patients are probably due to prolonged and/or repeated stress (26) rather than to the presence or intensity of pain. This hypothesis is consistent with the lack of significant correlation between clinical variables (TP count, FIQ, HAQ, VAS ratings of pain, fatigue, stiffness) and serum levels of NPY or HRV parameters in our FM group.

In this group, we also observed significant correlation between NPY levels and certain HRV variables, such as the SDNNDX and NN50. These results are also indicative of sympathetic hyperactivity. In other studies, however, FM patients have been found to have significantly decreased basal NPY levels that remain low even after 30 minutes on a tilt table (25). These observations support the opposite hypothesis, *i.e.* that FM-associated ANS dysfunction is characterised by stress-induced hyporeactivity. Further investigation is clearly

needed to define the actual significance of NPY in the pathogenesis of FM.

Finally, although ANS dysfunction is also a recognised feature of SSc, we found no significant difference between the serum NPY levels of our SSc group and those of the healthy controls.

It is important to remember that NPY is released by the postganglionic sympathetic neurons that innervate primary and secondary lymphoid organs, and it seems to play a crucial role in communication between the ANS and the immune system. NPY also seems to be implicated in the type 1 T-helper lymphocyte response, whereas the activated T cells found in SSc are predominantly of the type 2 T-helper lineage (27). This might explain why NPY levels in our SSc group were not significantly different from those of healthy subjects.

In conclusion, the findings presented above are all suggestive of crucial roles for autonomic dysfunction and NPY in the pathophysiology of FM. This is the first attempt to compare these parameters in FM and in another syndrome characterised by autonomic dysfunction, and it indicates that sympathetic activation in FM is stronger than that observed in SSc. Additional studies are necessary to define the complex roles played by NPY (and other neuropeptides) and the ANS in modulating pain and immunological functions in different diseases.

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