Correlation between nocturnal heart rate variability parameters and circulating neuropeptides in patients suffering from fibromyalgia

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Abstract

Objective

A consistent line of investigation proposes fibromyalgia (FM) as a stress-evoked, sympathetically maintained neuropathic pain syndrome. The purpose of this study was to measure dysautonomia-associated neuropeptide serum levels in women suffering from FM and to correlate these levels with heart rate variability parameters and disease severity.

Methods

We studied 23 women suffering from FM without comorbid conditions and 15 age and body mass index-matched healthy women. At the time of the study, all participants were free from medications that could affect the autonomic nervous system. Time-domain parameters were extracted from nocturnal (00.00 to 06.00 hours) heart rate variability Holter recordings. The following 7 neuropeptide levels were measured via the 7-Plex Human Neuropeptide Magnetic Kit Milliplex MAP: alpha-melanocyte-stimulating hormone, beta-endorphin, cortisol, neurotensin, orexin, oxytocin and substance P.

Results

The serum levels of beta-endorphin (669.24±186.28 vs. 541.71±146.26 pg/ml, p=0.028) and neurotensin (156.23±58.15 vs. 116.64±47.93 pg/ml, p=0.016) were significantly greater in patients with FM. Both neuropeptides were negatively correlated with the nocturnal heart rate R-R interval standard deviation (Rho=-0.52 p=0.025 and Rho=-0.6, p=0.003) and with the FIQR "tenderness to touch" (Rho -0.49 and Rho -0.41, p<0.05).

Conclusion

The serum levels of beta-endorphin and neurotensin in women suffering from FM correlate with nocturnal heart rate variability parameters, revealing sympathetic hyperactivity. The unexpectedly increased circulating levels of these two analgesic neuropeptides in the FM group can be interpreted as a homeostatic attempt by the central nervous system to ease peripherally generated pain.

Key words

neuropeptides, heart rate variability, fibromyalgia, dysautonomia

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Introduction

Fibromyalgia (FM) is a chronic, multisymptomatic disease characterised by widespread pain, fatigue, sleep disturbances, and dyscognition (1). Autonomic nervous system dysfunction may explain the multisystem features of FM. The autonomic nervous system is the main complex adaptive system of the body. Heart rate fluctuations are known to be determined by autonomic control mechanisms that can be studied via time-domain mathematical algorithms and spectral analysis of heart rate variability. Diverse groups of investigators have reported decreased heart rate variability in patients with FM (2, 3), indicating a state of sympathetic hyperactivity. Compared with 24-hour recordings, nocturnal heart rate variability analyses better discriminate patients suffering from FM from healthy controls (HC) (4).

A longstanding line of investigation suggests that FM is a stress-evoked, sympathetically maintained neuropathic pain syndrome (5). In contrast to HC, patients suffering from FM have norepinephrine-evoked pain (6). There is a clear relationship between FM and small-fibre neuropathy (7).

Neuropeptides are small proteinaceous substances produced by neurons that act on neuronal substrates. Although they are called neuropeptides because of their origin from neurons, unlike other peptides considered hormones, their effects are not necessarily carried out exclusively in the central nervous system. Within the periphery, neuropeptides can function similarly to peptide hormones and modulate almost all bodily functions. The modulation of functions by neuropeptides in target cells both in the periphery and at the brain level could suggest altered sensory perception (8).

There are few investigations on dysautonomia-associated circulating neuropeptides in patients with FM. The purpose of this study was to measure the serum levels of several dysautonomia-associated neuropeptides in women suffering from FM and to correlate their serum levels with nocturnal heart rate variability parameters and disease severity. Age-, sex-, and body mass index-matched healthy women were invited to participate in the control group.

Materials and methods

We studied 23 women with FM without comorbidities and 15 matched HC. At the time of the study, all participants were free from medications that could affect autonomic-related neuropeptide levels. All participants completed the Revised FM Impact Questionnaire (FIQR), Widespread Pain Index (WPI), Symptom Severity Scale (SSS), Polysymptomatic Distress Scale (PDS) and Small Fiber Neuropathy Symptoms Survey (SFNSS). All blood samples were obtained in the morning from fasted individuals. Tobacco and coffee were not allowed 24 hours before and during the procedures.

The levels of the following 7 neuropeptides were measured via the 7-Plex Human Neuropeptide Magnetic Kit Milliplex MAP (Merck-Millipore): alpha-melanocyte stimulating hormone (alfa-MSH), beta-endorphin, cortisol, neurotensin, orexin, oxytocin and substance P. Multiplex assays were carried out in a 96-well plate and analysed via a MagPix device. Serum samples were incubated with fluorescent magnetic beads conjugated with a specific antibody for each analyte. The beads were washed and incubated with a cocktail of soluble fluorescent antibodies against each analyte (MagPlex-C microspheres) and loaded into the instrument, after which the analytes were quantified (Milliplex Map, Luminex Corporation. Austin, Texas, USA).

Time domain parameters of heart rate variability were calculated from ambulatory 24-hour electrocardiographic recordings, and a sub-analysis of the same parameters was performed during the participants' hours of sleep at night (00.00 to 06.00 hrs). Measurements of heart rate variability followed international recommendations (9): mean of normal to normal R-R intervals (NN), standard deviation of the R-R interval (SDNN), standard deviation of the averages of R-R intervals in all 5 min segments of the entire recording (SDANN), the root mean square of successive differences between adjacent R-R intervals (RMSSD), standard deviation of differences between adjacent R-R intervals (SDSD) and the number of pairs of adjacent R-R intervals differing by

more than 50 milliseconds in the entire recording (NN50) divided by the total number of all R-R intervals (pNN50).
The protocol was approved by the institution review board (17-1010) and required written informed consent from the participants.

Statistical analysis
The Kolmogorov-Smirnov test was used to assess the normality of the data, Student's t test or the Mann-Whitney U-test was used for comparisons, and the Pearson or Spearman test was used for correlations. IBM SPSS v. 23.0 (IBM Corporation. Armonk, Nueva York, USA) was used for the analyses. Graphs were generated with GraphPad Prism v. 7.00 (GraphPad Software, Inc. San Diego, California, USA).

Results

Age and body mass index did not differ between the participants with FM (n=23) and the controls (n=15); as expected, all the clinimetric scales markedly differed between the groups (Table I).
Table II shows the serum levels of the 7 measured neuropeptides. Beta-endorphin (669.24±186.28 *vs*. 541.71±146.26 pg/ml, p=0.028) and neurotensin (156.23±58.15 *vs*. 116.64±47.93 pg/ml, p=0.016) levels were significantly higher in FM patients. Both neuropeptide levels were negatively correlated with the nocturnal heart rate R-R interval standard deviation (Rho -0.5 and Rho -0.6, p<0.05) and with FIQR "tenderness to touch" (Rho -0.4 and Rho -0.4, p<0.05) (Fig. 1, Table III). Beta-endorphin and neurotensin serum levels were highly correlated with each other in both the patient and HC groups (Rho = 0.939, p<0.0001).

Table I. Clinical scores of fibromyalgia patients (FM) and healthy controls (HC).

	FM n=23	HC n=15	*p*
Age	41 ± 8	40 ± 9	0.883
Body mass index	25 ± 3	26 ± 3	0.129
Years of disease duration	10 (5 - 16)	-	-
Visual analogue scale for widespread pain	74 (63 - 91)	2 (0-5)	< 0.0001
Tender points	17 (14 - 18)	3 (1- 6)	< 0.0001
Widespread pain index	14 ± 4	4 ± 4	< 0.0001
Symptom severity scale	9 ± 2	3 ± 2	< 0.0001
Polysymptomatic distress scale	23 ± 5	8 ± 5	< 0.0001
Revised fibromyalgia impact questionnaire	55 ± 21	5 ± 4	< 0.0001
Small fibre neuropathy symptoms survey	37 ± 16	7 ± 5	< 0.0001

Table II. Neuropeptide levels in fibromyalgia patients (FM) and healthy controls (HC).

	FM n=23	HC n=15	*p*
aMSH*	79 ± 36	120 ± 204	0.483
Endorphin*	669 ± 186	541 ± 146	0.028
Cortisol*	92767 ± 40488	97927 ± 31183	0.420
Neurotensin*	156 ± 58	116 ± 47	0.016
Orexin*	613 ± 78	687 ± 357	0.810
Oxytocin*	94 ± 46	129 ± 113	0.148
Substance P*	25 ± 6	27 ± 13	0.853

* Serum levels in picograms/millilitre.

Table III. Correlations between neuropeptides and time-domain parameters of heart rate variability in fibromyalgia patients (n=23).

Neuropeptide	Heart rate variability time domains	Spearman's Rho	*p*
Endorphin	SDNN night	-0.527	0.025
	SDANN night	-0.513	0.030
Neurotensin	SDNN 24 hours	-0.471	0.036
	SDNN night	-0.648	0.003
	SDANN night	-0.665	0.002
	SDSD night	-0.477	0.039
	pNN50 night	-0.468	0.043
	RMSSD night	-0.477	0.039

SDNN: standard deviation of the NN intervals; SDANN: mean standard deviation of the average NN intervals calculated over 5 minutes; SDSD: standard deviation of the successive NN differences; pNN50: percentage of adjacent pairs of RR intervals that differed by more than 50 milliseconds from each other; RMSSD: the square root of the mean of the sum of successive NN differences.

Discussion

Contrary to our expectations, the results of our study demonstrate that patients suffering from FM display increased serum levels of two analgesic neuropeptides, namely, beta-endorphin and neurotensin. Furthermore, the serum levels of both neuropeptides correlated with nocturnal heart rate variability deviances consistent with a state of sympathetic hyperactivity and with the key FM symptom 'tenderness to touch' assessed through the FIQR questionnaire.
Beta-endorphin and neurotensin are both involved in modulating pain, but they have distinct mechanisms of action and effects. Beta-endorphin is a neuropeptide with a powerful analgesic effect that is produced in the pituitary gland and exerts its action through distant opioid receptors. It produces analgesia by inhibiting the firing of peripheral somatosensory fibres. Beta-endorphin is released in response to stress and pain and has homeostasis-restoring capacity (10). We are not aware of previously published research measuring beta-endorphin serum levels in patients suffering from FM.
Neurotensin is a different analgesic neuropeptide that produces antinociceptive effects through nonopioid mechanisms that act on its specific receptors. In addition to its role in pain modulation, neurotensin regulates diverse dopaminergic and cholinergic autonomic functions (11, 12).
Our literature review revealed a single study in which serum neurotensin levels were measured in patients suffering from FM. Tsilioni *et al*. quantified several serum neuropeptide levels in patients with FM compared with an un-

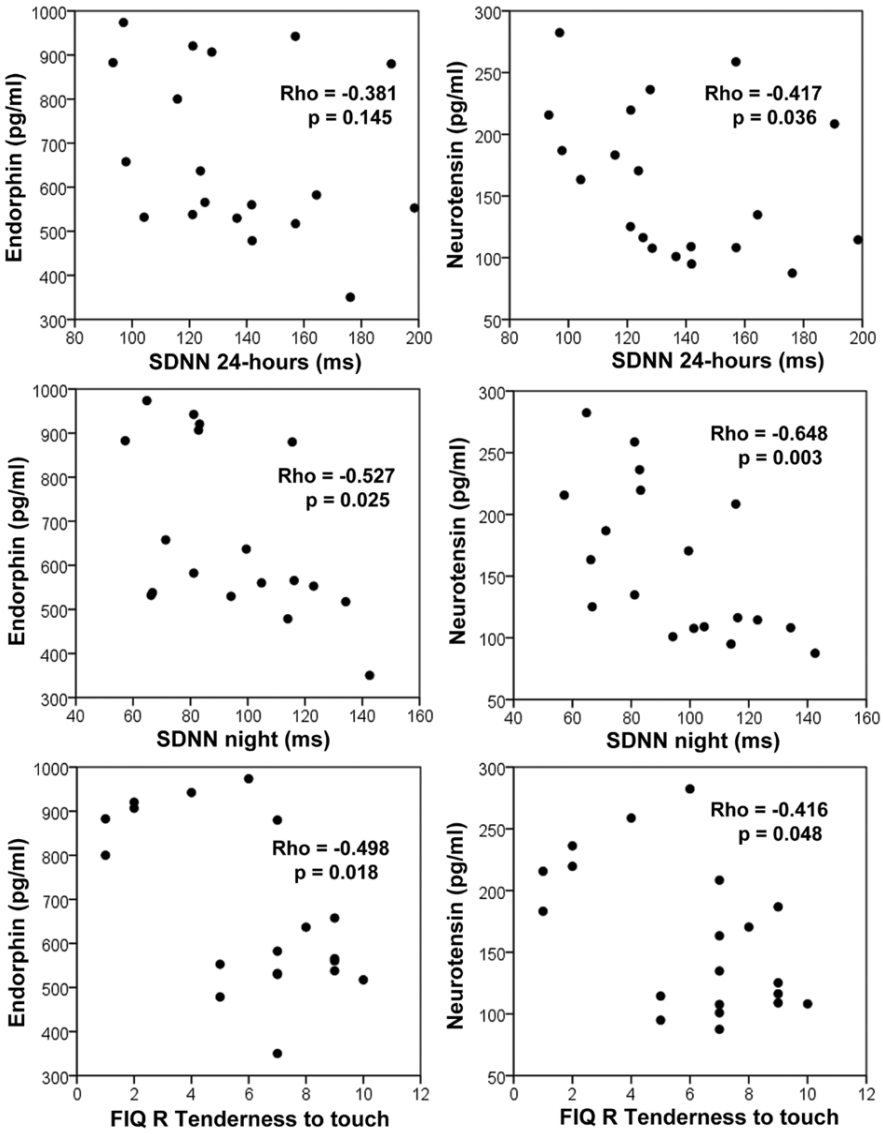

Fig. 1. Scatter plots of beta-endorphin and neurotensin representative correlations from fibromyalgia patients (n=23).

matched sex control group (all patients were women, whereas only 75% of controls were female). They mentioned that "there was no significant difference in serum levels of NT between FM patients and HC (data not shown)" (13).

Sleep difficulties are prominent in patients with FM. Prior work has demonstrated the advantage of measuring night-time (00.00 to 06.00 hours) heart rate variability over 24 hours to distinguish patients suffering from FM from HC, with the standard deviation of R–R intervals being the best for differentiating mathematical calculations (4).

Intriguingly, our results revealed that increased beta-endorphin and neurotensin serum levels were correlated with each other; moreover, the overexpression of both neuropeptides was interdependent on night-time heart rate variability abnormalities, revealing sympathetic hyperactivity. Beta-endorphin and neurotensin appear to play different stress response roles. Beta-endorphin promotes a global reduction in stress-related activity throughout the body (10); in contrast, neurotensin may induce anxiety and depression (14).

On the basis mostly of our prior research, we offer the following tentative explanation for the surprising neuropeptide results observed in the present investigation: we have suggested that FM is a stress-evoked, sympathetically maintained neuropathic pain syndrome. Diverse psychological, infectious and/or autoimmune stressors may induce dorsal root ganglia phenotypic changes with the ensuing development of neuropathic pain. The dorsal root ganglia contain the pain-transmitting small nerve fibre soma tightly encased by metabolically active satellite glial cells. There is a clear relationship between FM and small-fibre neuropathy (15). The increased circulating levels of beta-endorphin and neurotensin observed in the present investigation may be considered a homeostatic central nervous system response to peripherally originated painful stimuli. Obviously, more research is needed to clarify this issue.

Conclusion

The serum levels of beta-endorphin and neurotensin in women suffering from FM correlate with nocturnal heart rate variability parameters, revealing sympathetic hyperactivity. The unforeseen higher circulating levels of these two analgesic neuropeptides found in the FM group can be interpreted as a homeostatic attempt by the central nervous system to ease peripherally generated pain. However, the sample size of this pilot study is small, and studies with larger populations and different ethnicities are needed to reinforce or disprove the current findings.

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