Cardiac and Sympathetic Baroreflex Sensitivity Is Not Affected by Transcutaneous Vagus Nerve Stimulation in Hyperadrenergic Postural Tachycardia Syndrome

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Abstract

Hyperadrenergic postural tachycardia syndrome (HyperPOTS) is characterized by a shift of the sympatho-vagal balance toward sympathetic predominance. Transcutaneous vagus nerve stimulation (tVNS) might be beneficial in HyperPOTS, although the acute effects of tVNS on baroreflex sensitivity (BRS) are still unclear. We concomitantly evaluated BRS in the cardiac (cBRS) and sympathetic (sBRS) baroreflex arms in 19 HyperPOTS patients (39±11 yrs; 17 females) undergoing 75° head-up tilt test (HUT) during two randomized tVNS sessions with the device switched off and switched on. Variability of heart period (HP), systolic and diastolic arterial pressure (SAP and DAP), and muscle nerve sympathetic activity (MSNA) burst rate were extracted simultaneously from the ECG, arterial pressure and MSNA signals. cBRS and sBRS were estimated as the transfer function gain respectively from SAP to HP and from DAP to MSNA burst rate in the low (0.04-0.15 Hz) frequency band. cBRS significantly decreased during HUT but did not vary across tVNS sessions, while both HUT and tVNS had no impact on sBRS. We conclude that the acute effect of a single tVNS session on the sensitivity of different baroreflex arms is negligible in HyperPOTS, while HUT only seems to affect cBRS, potentially due to the HyperPOTS baseline sympathetic overactivity.

1. Introduction

Postural tachycardia syndrome (POTS) is a chronic form of dysautonomia, clinically characterized by inappropriate and sustained tachycardia upon standing (*i.e.*, an increase of more than 30 bpm in the first 10 minutes following postural change), in the absence of significant orthostatic hypotension [1]. It is estimated that its prevalence (up to 1% in developed countries [1]) makes it one of the most common forms of autonomic dysfunction, most commonly affecting women of childbearing age. Among the recognized subtypes of POTS, the hyperadrenergic class (HyperPOTS) [1,2] is characterized by symptoms of orthostatic intolerance compatible with a shift of the sympatho-vagal balance toward sympathetic predominance.

Transcutaneous vagus nerve stimulation (tVNS) [3] consists in the application of a low-voltage electrical current to anatomical regions with cutaneous afferents of the vagus nerve, with the aim of altering vagal activity. Preliminary studies on the acute effects of a single session tVNS on HyperPOTS patients suggest that it might reduce orthostatic intolerance symptoms [4] by modifying the baseline cardiac sympathetic overactivity. Baroreflex control is thought to be impaired in POTS [5]. However, it is unclear whether tVNS could have an impact on baroreflex sensitivity (BRS) in HyperPOTS, especially when considering different arms of the baroreflex. Indeed, baroreflex is the neural mechanism responsible for altering many physiological variables such as heart period (HP), muscle sympathetic nerve activity (MSNA) and peripheral resistances to limit variations of arterial

pressure (AP). We can therefore define the arm of the baroreflex that modifies HP according to AP changes as the cardiac baroreflex (cBR) [6] and estimate cBR sensitivity (cBRS) employing the spontaneous variability of systolic AP (SAP) and HP. Conversely, the arm of baroreflex that leads to changes in sympathetic nerve activity to buffer AP fluctuations is the sympathetic baroreflex (sBR) [7]. sBR sensitivity (sBRS) has been characterized by exploiting the spontaneous variability of diastolic AP (DAP) and MSNA [7], but an approach based on the changes of MSNA burst rate has also been suggested [8]. Different methodologies have been proposed for BRS assessment [9], among them a non-causal approach in the frequency domain estimates the BRS as the magnitude of the transfer function (TF) [10] computed between the variability series under analysis.

In the present work we propose a simultaneous evaluation on different arms of the baroreflex of the effects of tVNS in HyperPOTS [11], by means of TF gain (TFG) calculated from SAP to HP as an index of cBRS and from SAP to MSNA burst rate as a marker of sBRS.

2. TF Analysis

The BRS was assessed as the gain of the TF of the open loop input-output relationship between variability series [12]. Briefly, the cross-spectral density was estimated via bivariate autoregressive model [13] from SAP to HP for the assessment of cBR and from DAP to MSNA burst rate for the assessment of sBR. The model order was fixed to 10 and its coefficients were computed via traditional least squares approach. The TF was estimated as the ratio of the computed cross-spectral density from SAP to HP or from DAP to MSNA burst rate to the power spectrum of SAP or DAP. The squared coherence function $K^2(f)$ was computed as the ratio between the squared modulus of the cross-spectral density to the product of the power spectral densities of the two series. $K^2(f)$ ranges between 0 (null linear correlation) and 1 (maximum linear correlation). Both TFG and $K^2(f)$ were sampled at the frequency corresponding to the maximum of the $K^2(f)$ in the low frequency (LF, 0.04-0.15 Hz) band [9]. The resulting index was taken as BRS.

3. Experimental Protocol and Data Analysis

3.1. Experimental Protocol

Experimental procedures were performed at the Laboratory of Internal Medicine, Syncope Unit at Humanitas Research Hospital. Data were acquired from 19 HyperPOTS patients (39±11 yrs; 17 females) during two randomized sessions of electrical stimulation of the auricular branch of the transcutaneous vagus nerve, delivered to the right auricular cymba concha [3]. During the sham session the device (NEMOS®, Cerbomed, Erlagen, Germany) was switched off (OFF), while during the active session the device was switched on (ON) with stimulation pulse width set to 200 μs, the frequency to 25 Hz, and intensity adjusted between 0.1-6 mA, according to individual sensitivity. Electrocardiogram (ECG) from lead II, non-invasive AP (Nexfin monitor, BMEYE B.V., Amsterdam, Netherlands), and integrated MSNA obtained from microneurographic recordings of the activity of the peroneal nerve of the left leg (IOWA Nerve Traffic Analyzer 662C-3, University of Iowa Bioengineering, Iowa City, USA) were continuously recorded. Each tVNS session (i.e., OFF or ON) consisted of a 10-minute supine baseline (REST), followed by a graded head-up tilt (HUT) with an inclination of 75°. All signals were sampled at 1000 Hz. Patients were instructed to avoid intense physical activity, caffeine, smoking and alcohol in the 24 hours preceding the study and to perform a 3-day pharmacological washout for medication that might affect the autonomic nervous system. The study protocol adhered to the principles of the Declaration of Helsinki for medical research involving

Table 1. Time domain parameters assessed at REST and during HUT during OFF and ON tVNS sessions.

Parameter	OFF		ON	
	REST	**HUT**	**REST**	**HUT**
μ_{HP} [ms]	809.68±108.33	539.63±72.43*	753.20±104.10	604.03±111.24*
σ^2_{HP} [ms^2]	932.39±494.64	624.24±511.15	1006.11±559.34	921.42±868.95
μ_{SAP} [mmHg]	116.45±14.56	115.59±14.35	115.61±13.25	114.38±18.75
σ^2_{SAP} [mmHg^2]	10.58±6.90	38.43±21.68*	16.41±12.45	33.72±19.93*
μ_{DAP} [mmHg]	68.74±7.25	75.12±9.39	68.97±9.59	73.79±11.95
σ^2_{DAP} [mmHg^2]	4.11±2.89	18.35±13.70*	5.30±3.16	12.09±7.94*
μ_{MSNA} [bursts·s^{-1}]	0.61±0.17	0.65±0.19	0.52±0.22	0.68±0.22
σ^2_{MSNA} [bursts^2·s^{-2}]	0.05±0.01	0.07±0.04*	0.05±0.01	0.07±0.03*

REST: supine position; HUT: head-up tilt; OFF: stimulator off; ON: stimulator on; μ: mean; σ^2: variance; HP: heart period; SAP: systolic arterial pressure; DAP: diastolic arterial pressure; MSNA: muscle sympathetic nerve activity. The symbol * indicates $p<0.05$ REST vs HUT.

Table 2. cBR indexes assessed via TF method at REST and during HUT during OFF and ON tVNS sessions.

Parameter	OFF		ON	
	REST	HUT	REST	HUT
$K^2(LF)_{cBR}$	0.71±0.18	0.79±0.16	0.70±0.15	0.81±0.13
$TFG(LF)_{cBR}$ [ms·mmHg^{-1}]	14.20±9.87	3.96±3.07*	8.60±4.12	4.58±2.46*

REST: supine position; HUT: head-up tilt; OFF: stimulator off; ON: stimulator on; K^2: squared coherence; TF: transfer function; TFG: TF gain; LF: low frequency; cBR: cardiac baroreflex. The symbol * indicates $p<0.05$ REST vs HUT.

human subjects and was approved by the local Independent Ethics Board (authorization number: 2459). Written informed consent was obtained for all patients.

3.2. Series Extraction

R-wave peaks were identified from the ECG. Detected R-wave peaks were then visually checked for the presence of misdetection or arrhythmic beats. The *n*-th heart period (HP) was estimated as the time interval between two consecutive sinus R-wave peaks. The AP maximum within the *n*-th HP was identified as the *n*-th SAP value, and the following AP minimum was labelled as the *n*-th DAP value. MSNA bursts were automatically detected on the integrated MSNA signal [14]. To account for the baroreflex latency before a sympathetic response, MSNA bursts were searched in a defined temporal window (0.9-1.7 s) after the first R-wave peak delimiting the *n*-th HP [8]. The burst detection adaptive threshold was updated for each HP to follow baseline wandering and physiological variations of the MSNA burst amplitude [14], and peaks surpassing the current threshold value were labelled as MSNA bursts. The burst rate of MSNA was then calculated as the number of identified bursts over a moving window of 5 s. The resulting stepwise count MSNA signal was then low-pass filtered (cutoff frequency: 0.5 Hz) and then sampled at the occurrence of the cardiac beat to obtain a MSNA burst rate series synchronous with HP, SAP and DAP [8]. Sequences of 300 consecutive values were selected. Due to signal quality, the MSNA burst rate series had to be discarded for 3 patients at REST and 6 during HUT. Mean (μ) and variance (σ^2) were computed for HP, SAP, DAP and MSNA burst rate variability series, labelled as μ_{HP}, σ^2_{HP}, μ_{SAP}, σ^2_{SAP}, μ_{DAP}, σ^2_{DAP}, μ_{MSNA}, σ^2_{MSNA}, and expressed in ms, ms^2, mmHg, mmHg^2, mmHg, mmHg^2, bursts·mmHg^{-1}, bursts^2·mmHg^{-2}, respectively.

3.3. Statistical Analysis

Normality of data was verified via Shapiro-Wilk test. Two-way analysis of variance (Holm-Sidak test for multiple comparisons) was performed to evaluate the HUT-induced changes within the same experimental session (*i.e.*, OFF or ON) and the effects of tVNS within the same experimental condition (*i.e.*, REST or HUT). Results are presented as mean±standard deviation. Statistical analysis was carried out using a commercial statistical program (Sigmaplot, v.14.0, Systat Software, Inc., Chicago, IL, USA). A $p<0.05$ was always deemed significant.

4. Results

Table 1 reports temporal indexes for the HP, SAP, DAP and MSNA burst rate series. μ_{HP} decreased with HUT regardless of type of tVNS. No significant change of σ^2_{HP}, μ_{SAP}, μ_{DAP} or μ_{MSNA} was observed with HUT and tVNS, while σ^2_{SAP}, σ^2_{DAP}, σ^2_{MSNA} significantly increased following HUT. Table 2 summarizes the results obtained from cBRS analysis. $TFG(LF)_{cBR}$ decreased following HUT in both ON and OFF sessions, while $K^2(LF)_{cBR}$ did not vary with either HUT or tVNS. Table 3 summarizes the results obtained from sBRS analysis. No difference was detected across any experimental session or condition for $K^2(LF)_{sBR}$ and $TFG(LF)_{sBR}$.

5. Discussion

The main findings of this study can be summarized as follows: i) HyperPOTS exhibited a response to HUT compatible with the pathology; ii) cBRS and sBRS showed different responses to HUT; iii) tVNS did not produce acute effects on cardiovascular control, sympathetic activity or BRS in HyperPOTS.

HUT is the preferred diagnostic test to evaluate symptoms of POTS and was applied in this study to monitor baroreflex control. Our study confirmed trends in time domain indexes reported in previous studies [2]. HyperPOTS is known [2] to exhibit a decrease of μ_{HP}, and an increase of σ^2_{SAP} and σ^2_{DAP} during HUT indicating a sympathetic activation. Direct measures of sympathetic activity supported this conclusion. Indeed, μ_{MSNA} tended to augment with HUT, while the increase of σ^2_{MSNA} was significant [8]. Regarding baroreflex function, cBRS is thought to be altered in POTS [5]. However, responses to HUT were similar to those reported in previous studies in healthy young subjects [11], with a decrease of cBRS and a stable sBRS in the LF band following HUT. The different responses to HUT of the two arms highlight the importance of a concurrent assessment of both cBRS and sBRS to investigate pathophysiological mechanisms of the baroreflex and the complementarity of the different mechanisms involved in the regulation of AP [11].

Table 3. sBR indexes assessed via TF method at REST and during HUT during OFF and ON tVNS sessions.

Parameter	OFF		ON	
	REST	**HUT**	**REST**	**HUT**
$K^2(LF)_{sBR}$	0.32±0.21	0.48±0.32	0.40±0.24	0.52±0.35
$TFG(LF)_{sBR}$ [bursts·s^{-1}·mmHg^{-1}]	0.08±0.05	0.06±0.03	0.08±0.05	0.07±0.04

REST: supine position; HUT: head-up tilt; OFF: stimulator off; ON: stimulator on; K^2: squared coherence; TF: transfer function; TFG: TF gain; LF: low frequency; sBR: sympathetic baroreflex. The symbol * indicates $p<0.05$ REST vs HUT.

Preliminary studies on the acute effects of a single tVNS session in POTS [4] found an improvement in patients with low baseline vagal modulation (*i.e.*, compatible with a diagnosis of HyperPOTS subtype), as assessed from HP variability in response to graded HUT, and a better orthostatic tolerance in terms of minutes of HUT tolerated. However, to our knowledge this is the first study characterizing BRS in a specific subtype of POTS, namely the HyperPOTS group, especially when considering simultaneously the sBR and cBR arms. Previous studies [15] found a significant response to tVNS compared to sham stimulation in healthy young men in terms of cBRS assessed as the slope of the sequences of concordant variations of HP and SAP. The same effect does not seem to hold in HyperPOTS, as well as tVNS seemingly not affecting sBRS directly. This result might be related to the baseline sympathetic hyperactivity, causing a blunted response to HUT [2] that cannot be acutely resolved by an individual non-pharmacological intervention such as tVNS acting specifically on the vagal modulation. We conclude that a single tVNS session is not sufficient to acutely affect cBRS and sBRS in HyperPOTS, and that repeated, constant use of tVNS might be necessary to provoke more chronic modifications of vagal modulation that might affect baroreflex function. Future studies should focus on the chronic effect of long term tVNS in HyperPOTS, and on the analysis of different subtypes of POTS patients.

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