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Correlation between sonographic morphology and function of the cervical vagus nerves☆

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ABSTRACT

The heart receives parasympathetic and to a lesser degree sympathetic input via the vagus nerve. Here, we investigated whether morphological changes of the cervical vagus nerves (VN) as assessed by high-resolution ultrasound (HRUS) correlated with the autonomic cardiac innervation. Measurement of heart rate variability (HRV) and HRUS of the VNs were performed in 88 healthy subjects (50 female; mean age 56 ± 18 years). HRV parameters and the cross-sectional area (CSA) of the VNs correlated both inversely with age. We also found an inverse correlation between the left VN-CSA and HRV as well as parasympathetic parameters. The results imply an asymmetric parasympathetic (vagal) innervation of the heart.

1. Introduction

Unlike for peripheral nerves, the examination of the autonomic nervous system is currently limited to mainly functional testing (Novak, 2011; Freeman and Chapleau, 2013), since the direct visualization of sympathetic or parasympathetic nerve fibers is either invasive, as in the case of skin biopsies, or requires nuclear medicine imaging (Travin, 2013).

The physiological regulation of the heart rate is determined by the activity of the autonomic nervous system and can be evaluated by the assessment of the heart rate variability (HRV). Different parameters of the HRV are predominantly associated with either the sympathetic or the parasympathetic activity (Task Force, 1996). The cardiac parasympathetic efferent fibers originate from the nucleus ambiguous and to a lesser extent from the dorsal motor nucleus (DMN) (Geis et al., 1981). Together with the efferent and afferent axons of the solitary nucleus they form the vagus nerve (VN) complex. Furthermore, the vagus nerve also includes afferent axons which converge onto the spinal trigeminal nucleus. High-resolution ultrasound (HRUS) as a bedside imaging method allows the reliable visualization of the cervical VNs and the measurement of even small changes of their cross-sectional areas (CSA) (Pelz et al., 2018a). Currently, it is unclear whether morphological changes of the VNs are accompanied by functional changes.

Therefore, the aim of this study was to examine whether the morphology of the cervical portion of the VNs, namely their CSA, correlates with the parasympathetic activity as assessed by HRV in healthy subjects.

2. Material and methods

The study was approved by the local ethics committee (reference no.: 251-15-13072015). All participants provided informed consent prior to being included in the study.

2.1. Demographic and clinical data

Eighty-eight healthy subjects (50 female, mean age 56 ± 18 years (range 23–80 years), mean weight 73 ± 14 kg (range 45–110 kg), mean height 170 ± 9 cm (range 145–190 cm)) participated in this study. Exclusion criteria were any neurological disease, a history of neck surgery and any kind of cardiac arrhythmia. All participants underwent a thorough neurological examination to exclude in particular those with a clinically detectable pre-existing polyneuropathy.

2.2. High-resolution ultrasound and analyses of the ultrasound images

All 88 subjects were examined with HRUS by an experienced nerve sonographer (JP, IM, TBW) using the Esaote MyLab Five system with a

Abbreviations: HRV, heart rate variability; VN, vagus nerve; CSA, cross-sectional area; HRUS, high resolution ultrasound; DMN, dorsal motor nucleus; SA, sinoatrial; AV, atrioventricular

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15 MHz transducer (probe LA435) as described previously (Pelz et al., 2018a). Briefly, all participants were in the supine position with the sonographer sitting behind them holding the ultrasound transducer in an axial position. The settings of the ultrasound systems were individually optimised with respect to gain, depth, and focus. Subsequently, each VN was visualized at the level of the thyroid gland where it was located within the carotid sheath and could clearly be differentiated from the surrounding tissue. Three native B-mode images were recorded at each side. All measurements were done offline using Viewpoint (5.6.25.281, General Electric Company). Image analysis was done in a blinded fashion, i.e. the assessor was unaware of the subject's identity and the side of the VN. To assess the VN-CSA, its contour within the hyperechoic epineural rim was outlined manually with the Viewpoint software (5.6.25.281, General Electric Company). The median of the three VN-CSA measurements was used for statistical analyses.

2.3. Assessment of heart rate variability

After at least 5 min of rest, RR intervals were measured on electrocardiograms at normal breathing for 10 min under resting and in supine position each via the computer-based system ProSciCard (MedSet Medizintechnik GmbH, Hamburg, Germany). Besides heart rate at rest, several indices of the HRV were automatically computed: the standard deviation of RR-intervals (SDRR, higher index indicates higher variability), the root mean square of successive differences (RMSSD, estimate of short-term components of the HRV), the number of all RR-intervals/maximal frequency (HRV index, higher index indicates higher variability). The SDRR and the HRV index were considered as a marker for the overall HRV and the RMSSD as a marker for the parasympathetic activity (Task Force, 1996). In addition, power spectral analyses in the low (ln(LF), 0.05–0.15 Hz) and in the high frequency spectrum (ln(HF), 0.15–0.5 Hz) were performed and the low frequency/high frequency ratio (ln(LF/HF) ratio) was calculated. While power spectral analysis in the low frequency spectrum rather represents the sympathetic activity, power spectral analysis in the high frequency spectrum is supposed to correlate with the parasympathetic (or vagal) activity and, thus, their ratio is supposed to represent the sympathovagal balance.

2.4. Statistical analysis

Statistical analyses were performed with SPSS version 24.0 (IBM Corporation; New York, NY, USA). Explorative analysis of the HRV parameters revealed extreme outliers in the power spectral analyses which lacked biological plausibility. Therefore, extreme outliers were excluded based on Tukey's hinges (first quartile $-3*$interquartile range (IQR) and third quartile $+3*$IQR), visualized in boxplots (Krzywinski and Altman, 2014). A multiple linear regression analysis was calculated to examine the selective impact of demographic data (age, height, weight, gender) and the right and left VN-CSA on every HRV parameter. A $p \leq 0.05$ was considered statistically significant.

3. Results

The mean CSA of the right VN was $2.4 \pm 0.6\,mm^2$ and $1.8 \pm 0.4\,mm^2$ for the left VN (Wilcoxon, $p < 0.001$). There was an inverse correlation between age and VN-CSA (Pearson: right side -0.26, $p = 0.015$; left side -0.24, $p = 0.022$). We also found a strong inverse correlation between age and time-dependent as well as frequency-dependent HRV parameters (Table 1). Moreover, the left VN-CSA *inversely* correlated with global HRV parameters (SDRR, HRV index) as well as parameters of parasympathetic activity (RMSSD, ln(HF)) (Table 1). This *inverse* correlation between the left VN-CSA and global HRV parameters as well as parameters of parasympathetic activity persisted when demographic data and in particular age were considered as co-factors. No correlations were found between the left VN-CSA and sympathetic parameters as well as the right VN-CSA and any HRV parameter.

4. Discussion

This HRUS study examined whether morphological changes of the VNs are accompanied by autonomic changes as assessed by the HRV and its derivatives. In agreement with recent studies we found an inverse correlation between age and VN-CSA (Pelz et al., 2018a) and age and HRV parameters (Antelmi et al., 2004).

Differently than expected, there was an *inverse* correlation between the left VN-CSA and global HRV parameters as well as parameters of parasympathetic activity when demographic data and in particular age were considered as co-factors. Therefore, decrease of HRV with age is attenuated when the decrease of the left (but not the right) VN-CSA is comparatively larger.

With increasing age the parasympathetic control of the heart declines. As a consequence, the relative contribution of sympathetic activity increases. Overall this results in a decrease of global and parasympathetically modulated HRV parameters (De Meersman and Stein, 2007; Abhishekh et al., 2013).

As demonstrated in the current study in healthy subjects and recently in patients with Parkinson's disease (Pelz et al., 2018b), there is a morphological asymmetry between both VNs with the right VN being larger than the left one. This may be in part the result of the vagal innervation of unpaired abdominal organs with the right VN predominantly innervating parts of the small intestine and the colon and the left VN sending branches to the stomach, the liver and the superior part of the duodenum (Al-Kureischi, 1979). In addition, this asymmetry may also represent the morphological correlate of the asymmetric cardio-vagal innervation. So far, this lateralized cardio-vagal innervation was only addressed in animal studies. In different species up to 70% of cardiac preganglionic neurons were located in the external formation of the nucleus ambiguous and about 20% in the DMN (Geis et al., 1981). In an anterograde tracing study in rats, the ganglionic plexus related to the sinoatrial (SA) node and the ganglionic plexus related to the atrioventricular (AV) node received efferent fibers from both DMN. However, the right DMN sent more fibers to the ganglionic plexus related to the SA node while the left DMN preferably projected to the ganglionic plexus related to the AV node (Cheng et al., 1999). Accordingly, stimulation of the right VN inhibited the SA node while left vagal stimulation affected AV nodal conduction (Hamlin and Smith, 1968). These data from animal studies are supported by electrophysiological studies in humans. The transcutaneous stimulation of the right auricular branch of the VN tended to increase the HRV index and ln(HF) which indicates a rise in parasympathetic activity whereas left auricular branch stimulation rather tended to decrease ln(HF) (Weise et al., 2015). Accordingly, de Couck and co-workers demonstrated an increase of SDRR only when stimulating the right auricular branch of the VN. However, power spectral analyses in the low and in the high frequency spectrum were similar after right and left transcutaneous stimulation of the VN's auricular branch (de Couck et al., 2017). Thus, in order to avoid cardiac events VN stimulation is performed on the left side for the therapy of drug-refractory epilepsy (Panebianco et al., 2015). It has even been suggested that cardiovascular diseases such as chronic heart failure are associated with an imbalance in the cardiac autonomic nervous system with increased sympathetic and decreased parasympathetic (vagal) activity (Hauptman et al., 2012). Therefore, for the treatment of chronic heart failure where the maximum stimulation effect on the cardiac autonomic system is desired the right VN is stimulated (De Ferrari et al., 2011). This asymmetric effect of VN stimulation seems to be predominantly a parasympathetic effect and might follow from an asymmetric parasympathetic but perhaps also from an asymmetric sympathetic innervation of the heart. Noteworthy, both VN contain a relevant amount of sympathetic fibers (Seki et al., 2014; Verlinden et al., 2016). However, it is unclear whether the

Table 1
Multiple linear regression analyses to examine the selective impact of demographic data and right and left vagus nerve cross-sectional area (VN-CSA) on the parameters of the heart rate variability (HRV). Beta-coefficient and *p*-values (in brackets); significant findings are bold. ln(*HF*) power spectral analysis in the high (0.15–0.5 Hz) frequency spectrum, ln(*LF*) power spectral analysis in the low (0.05–0.15 Hz) frequency spectrum.

	Heart rate at rest	SDRR	RMSSD	HRV index	ln(HF)	ln(LF)	ln(HF/LF)
Age	−0.063 (0.614)	**−0.506 (< 0.001)**	**−0.373 (0.001)**	**−0.632 (< 0.001)**	**−0.445 (< 0.001)**	**−0.582 (< 0.001)**	−0.065 (0.602)
Height	0.138 (0.504)	−0.024 (0.824)	0.091 (0.416)	0.018 (0.854)	0.129 (0.215)	0.107 (0.292)	0.072 (0.569)
Weight	0.018 (0.889)	0.0 (0.998)	−0.151 (0.195)	−0.056 (0.584)	**−0.237 (0.026)**	−0.106 (0.302)	0.120 (0.349)
Gender	0.087 (0.441)	0.009 (0.929)	0.104 (0.298)	−0.021 (0.815)	0.176 (0.059)	0.027 (0.767)	−0.027 (0.808)
Right VN-CSA	0.113 (0.443)	0.104 (0.422)	0.054 (0.676)	−0.021 (0.856)	0.186 (0.126)	0.059 (0.618)	0.081 (0.587)
Left VN-CSA	−0.076 (0.605)	**−0.349 (0.007)**	**−0.269 (0.041)**	**−0.245 (0.035)**	**−0.263 (0.031)**	−0.111 (0.345)	0.088 (0.551)

sympathetic vagal fibers are running to the heart or rather to abdominal organs (Bonaz et al., 2017). Moreover, the overall sympathetic cardiac innervation is mainly provided by the cervical and thoracic cardiac nerves branching off the sympathetic chain.

There are several limitations. At first, we only examined healthy subjects. Further studies in patients with diseases of the autonomic nervous system like diabetic or inflammatory polyneuropathies that are known to impair the HRV (Kuehl and Stevens, 2012) as well as patients with chronic heart failure are needed. These studies should address whether this association between morphology and function of the (left) VN is also evident under pathologic conditions. Grimm and co-workers demonstrated that patients with Guillain-Barré syndrome with autonomic dysfunction had a significantly increased VN-CSA compared to patients without autonomic dysfunction (Grimm et al., 2016). However, it remains unclear whether they sonographed one or both sides. Furthermore, we only measured the HRV as a surrogate for the parasympathetic activity. The parasympathetic cardiac innervation is mainly determined by two of the four vagal brainstem nuclei. Future studies should examine further vagal-mediated autonomic, mainly gastrointestinal functions.

Finally, although time-dependent as well as frequency-dependent HRV analyses are routinely performed to examine the impact of the autonomic nervous system on cardiac control in healthy subjects and patients, they might not be sufficient to evaluate sympathetic function comprehensively. For instance, Eckberg critically appraised the concept of assessing sympathovagal balance by frequency-dependent HRV analyses and in particular that lower-frequency RR-interval rhythms (ln (LF)) mainly reflected sympathetic nerve traffic to the heart while higher-frequency RR-interval rhythms (ln(HF)) were mediated almost exclusively by fluctuations of vagal-cardiac nerve activity. Reviewing the literature, he even concluded that 'vagal contributions to baseline lower-frequency RR-interval fluctuations were great, and evidence that baseline lower-frequency RR-interval spectral power was related quantitatively to sympathetic-cardiac nerve traffic was nonexistent' (Eckberg, 1997). This has to be kept in mind when interpreting our and HRV data in general.

5. Conclusion

We found an inverse correlation between age as well as the left VN-CSA with HRV and parasympathetic cardioautonomic activity which underlines the assumption of an asymmetric parasympathetic (vagal) innervation of the heart.

Declaration of Conflicting Interest

Nothing to report.

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