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INPUT FOR BAROREFLEX ANALYSIS: WHICH BLOOD PRESSURE SIGNAL SHOULD BE USED?

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The baroreflex (BR) is an important physiological regulatory mechanism which reacts to blood pressure perturbations with reflex changes of target variables such as the heart period (electrocardiogram derived RR interval) or the peripheral vascular resistance (PVR). Evaluation of cardiac chronotropic (RR as a target variable) and vascular resistance (target PVR) BR arms was in previous studies mainly based on the use of the spontaneous variability of the systolic or diastolic blood pressure (SBP, DBP), respectively, as the input signals. The use of other blood pressure measures such as the mean blood pressure (MBP) as an input signal for BR analysis is still under investigation. Making the assumption that the strength of coupling along the BR indicates the more appropriate input signal for baroreflex analysis, we employ partial spectral decomposition to assess in the frequency domain the causal coupling from SBP, MBP or DBP to RR or PVR. Noninvasive beat-to-beat recording of RR, SBP, MBP and DBP and PVR was performed in 39 and 36 volunteers in whom orthostatic and cognitive loads were evoked respectively through head-up tilt and mental arithmetic task. At rest, the MBP was most tightly coupled with RR, in contrast to the analysis of the vascular resistance BR arm where the results showed similar importance of all blood pressure input signals. During orthostasis, the increased importance of SBP as the input signal for BR analysis along the cardiac chronotropic arm was demonstrated. In addition, the gain from MBP to RR was more sensitive to physiological state changes compared to gains with SBP or DBP signal as inputs. We conclude that the coupling strength depends not only on the analysed baroreflex arm but also on the selection of the input blood pressure signal and the physiological state. The MBP signal should be more frequently used for the cardiac baroreflex analysis.

Key words: *arterial baroreflex, blood pressure, cardiovascular variability, spectral analysis, transfer function, head-up tilt, mental arithmetic task, muscle sympathetic nerve activity*

INTRODUCTION

The baroreflex (BR) is a very important cardiovascular feedback control mechanism maintaining the optimal blood pressure level required to accommodate a particular task or condition (posture changes, cognitive load, exercise, *etc*.) through the modulation of heart rate (cardiac chronotropic BR arm), cardiac contractility (cardiac inotropic BR arm), peripheral vascular resistance (vascular resistance BR arm), or venous tone (venous arm) (1-5). During the past decades, several studies have shown that changes in the characteristics of the baroreflex function reflect alterations in cardiovascular neural control and could have an important diagnostic and prognostic value in a variety of cardiovascular disorders, including myocardial infarction, orthostatic intolerance and cardiac failure (1, 2, 4, 6-10).

Even though the BR represents a complex mechanism involving several reflex control mechanisms operating along different arms, due to the demanding process of measurement of the physiological output variables its evaluation was mainly focused on the cardiac chronotropic arm and - more rarely - on the vascular resistance arm. As a common practice, since the introduction of the quantitative method of assessing the baroreflex sensitivity related to the cardiac chronotropic arm, the systolic blood pressure (SBP) and the heart rate (or its reciprocal value, *i.e*. the RR interval measured from the electrocardiogram) have been extensively used as the input (source) and output (target) for BR analysis based on spontaneous cardiovascular variability (11-14). Hence, although Arndt *et al*. (15) found high positive correlations not only between systolic but also between mean or diastolic blood pressure values and the average firing rate of the baroreceptor afferent traffic measured in anaesthetized cats, the SBP is preferably used as the input instead of other variables that can be extracted from the blood pressure waveform (*e.g.* diastolic (DBP) or mean blood pressure (MBP) (16)).

Regarding the vascular resistance BR arm representing the second most assessed baroreflex arm, the choice of input and output signal for BR analysis was more diverse. In the majority of previous studies, the invasive recording of muscle sympathetic nerve activity (MSNA) from the peroneal nerve (17-20) was used as an output signal. Despite the fact that muscle sympathetic nerve activity measured *via* microneurography could be regarded as the 'gold standard' for assessment of sympathetic outflow in humans,

its application has several limitations: the output signal comes only from a limited portion of the circulation, the procedure is invasive and it is commonly associated with the high intraindividual variability reflecting the challenge of reliable locating and maintaining the ideal electrode placement for recording requiring experienced measuring person (21-24). Alternatively, the non-invasive beat-to-beat recording of peripheral vascular resistance (PVR) (3, 25-27) or pulse transit time (28) was also considered as an output for this arm. The most frequently used input signal for vascular resistance BR analysis was DBP (12, 14, 17, 19, 29-32) rather than the MBP (33) or the SBP (3, 34). The prevalent use of DBP as the input was usually justified by the results of Sundlof and Wallin (35), where a strong inverse relationship between spontaneous fluctuations of MSNA and DBP was observed, although only slightly weaker correlations were also found with the MBP and pulse pressure in the same study.

Taken together, the previous studies suggest that SBP and DBP variability are commonly used as the input signals respectively to assess the cardiac chronotropic and the vascular resistance BR arms, while the use of alternative blood pressure measures was not sufficiently explored. We suggest that more careful analysis, focused on a strategic selection of the input signal for the assessment of specific BR arms, is needed for a more appropriate baroreflex analysis (36). Accordingly, this study aims to assess comparatively the strength of coupling along the cardiac chronotropic and vascular resistance BR arms, as well as the gain of the reflex, investigated taking respectively RR interval and PVR variability as the output signals, using either SBP, MBP or DBP as input signals. To the best of our knowledge this is the first study comparing the strength of causal coupling along the cardiac chronotropic and vascular resistance BR arms using three basic beat to beat blood pressure measures as the input signal. To assess the BR during different physiological conditions, the analysis was performed in healthy subjects monitored in a resting state and during physiological stress induced by orthostatic and cognitive challenges.

Preliminary data were presented at the 11th Conference of the European Study Group on Cardiovascular Oscillations (37).

MATERIALS AND METHODS

Study group

In this study, the baroreflex response to orthostatic and cognitive challenges to the cardiac chronotropic and vascular resistance BR arms was evaluated in two groups of young healthy volunteers. The effect of orthostatic stress was assessed in 39 volunteers (22 women; age: 19.4 (2.3) years (arithmetic mean (standard deviation)), range: 16.0–25.4 years, BMI: 22.1 (2.5) kg/m^2) and the effect of cognitive load was evaluated in 36 volunteers (21 women; age: 19.6 (2.5) years, range: 16.0–25.4 years, BMI: 21.9 (2.3) kg/m^2). The exclusion criteria included: cardiovascular, respiratory, or other diseases (diabetes mellitus, obesity, psychiatric disorders) that could alter autonomic and vascular functions.

This study was approved by the Ethics Committee of the Jessenius Faculty of Medicine, Comenius University, and all participants or their parent (guardian) gave their written informed consent before participation.

Experimental protocol

Data collection was carried out between 8 AM and 11 AM. All participants were asked to refrain from drinking caffeinated, alcoholic, or energetic beverages and from performing heavy exercise for 24 hours before the examination. The study protocol consisted of four phases (*Fig. 1*). After 15 minutes in a supine position, mild orthostatic stress was evoked by tilting the subjects on the motor-driven tilt table to 45 degrees (head-up tilt (HUT), 8 minutes); this phase was followed by a phase of supine recovery lasting 10 minutes to allow physiological parameters to recover to a baseline level. All subjects were able to complete the HUT without showing signs of presyncope. Then, a mild cognitive load was evoked in the last phase by executing a simple nonverbal mental arithmetic task in the supine position (MA, 6 minutes). During the MA task, all participants were instructed to sum the digits of randomly selected three-digit numbers until the one-digit number was reached (if the sum of three digits resulted in two-digit number, these two digits had to be summed up) and to decide if the final result was odd or even by clicking a wireless computer mouse on the corresponding push button projected on the ceiling. During the MA task, the subject was disturbed by the rhythmical noise of the metronome and instructed to perform the task as quickly as possible with a minimal error rate. The participants were instructed to refrain from movements or speech for the whole duration of the examination. The subjects breathed spontaneously and did not make any effort to regulate their breathing rate or tidal volume. All participants breathed with a frequency within the high frequency (0.15–0.5 Hz) oscillations band (minimal breathing rates during the four phases of the examination protocol were 0.25 Hz). More detailed description of the experimental protocol is presented elsewhere (3, 38, 39).

Data acquisition

We continuously non-invasively measured beat-to-beat values of heart period (beat-to-beat values of RR interval calculated as the temporal distance between two consecutive R peaks) from the electrocardiogram (ECG, CardioFax ECG-9620, NihonKohden, Japan), systolic (represented by the maximum value of the arterial pressure waveform (*i.e. SBP(n)*) inside the *n*th cardiac cycle (*i.e. RR(n)*)), mean (*i.e. MBP(n)*, calculated as the average of the blood pressure waveform taken between the current and the next cardiac

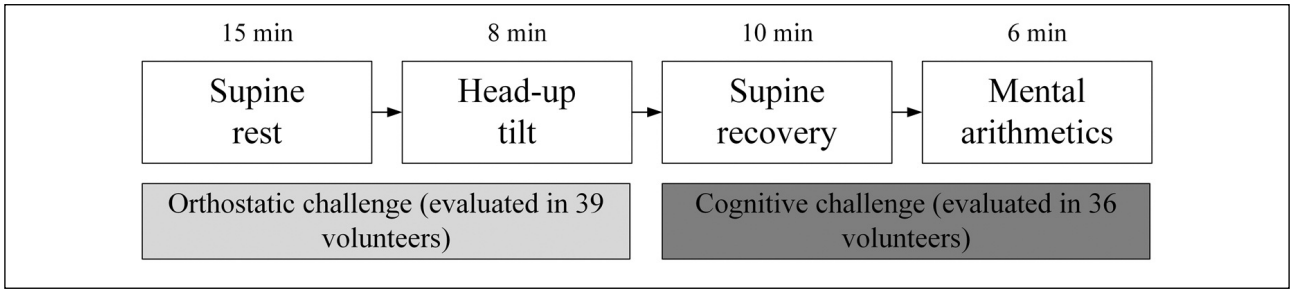

Fig. 1. Diagram of the experimental protocol.

upstroke) and diastolic blood pressure (*i.e. DBP(n)*, the minimum value of the arterial pressure waveform following the n^{th} cardiac cycle) from the blood pressure signal recorded by the photoplethysmographic volume-clamp method (Finometer Pro, Finapres Medical Ssystems BV, Amsterdam, Netherlands). Cardiac output (CO) was derived as:

$$CO(n) = 60 \cdot \frac{SV(n)}{RR(n-1)} [L/min] \quad (1)$$

where SV denotes the stroke volume, calculated using the Bernstein and Sramek formula from the impedance cardiography signal (CardioScreen® 2000, Medis, Ilmenau, Germany) (40).

Synchronous time series of 300 beats were selected for the five variables in each phase of the experimental protocol. In order to allow stabilization of probands within each phase and to exclude the transient non-stationary parts of the recordings mostly occurring after the protocol phase change, the 300 beats lasting segments used for the data analysis started 8 minutes after the beginning of the supine rest phase, 3 minutes after the starting of HUT and supine recovery phase, and 2 minutes after starting the MA task. The missing beat-to-beat values of CO were interpolated by cubic spline (we allowed a maximum of 15 missing values in 300 beats long recording and no more than 4 in a row, otherwise the recording was excluded). The PVR was calculated for each heartbeat as the ratio of the MBP and CO:

$$PVR(n) = MBP(n)/CO(n) \ [mmHg.min.L^{-1}]. \quad (2)$$

Data analysis

The quantification of the spectral causal coupling and spectral gain (sensitivity) along the BR, reflecting respectively the effectiveness of the reflex (the strength (closeness) of the relation between output signal and the blood pressure input signal) and the responsiveness of the BR to blood pressure variations (the magnitude of the output signal response to unit blood pressure change), was carried out in the frequency domain using partial spectral decomposition (3, 41-43). This method is based on the linear parametric representation of bivariate autoregressive processes (in our case, the input and output variability series chosen for each analysis) and provides a frequency-specific evaluation of the strength of the spectral causal coupling and of the spectral gain from the input to the output series. For the analysis of cardiac chronotropic and vascular resistance BR arms, the SBP, MBP or DBP were taken as source (input) signals and RR and PVR as target (output) signals, respectively. While the linear parametric model yielding the spectral measures of coupling and gain typically describes time lagged effects between the two analysed series, setting instantaneous (*i.e.* non-delayed) effects is also important to account for fast (within-beat) control mechanisms (3, 41, 42). In this work, the measurement convention, illustrated in *Fig. 2*, allows to set instantaneous effects from *SBP(n)* and *MBP(n)* to *RR(n)*, from *RR(n)* to *DBP(n)* and from *PVR(n)* to *SBP(n)*, *MBP(n)* or *DBP(n)*. Based on the rationale that the most proper setting for the analysis is the one highlighting the effectiveness of the investigated reflex, we assume that a higher spectral causal coupling strength indicates a more appropriate signal pair for baroreflex analysis. The causal analysis was performed in the low frequency (LF, 0.04 to 0.15 Hz) band, which, as shown in previous studies (25, 43-46), is appropriate for investigating the reflex avoiding the confounding effects of respiration which typically occur at higher frequencies. Details on data pre-processing and analysis are presented in our previous study (3).

Statistical analysis

For each analysed distribution, the data normality was assessed by the Shapiro-Wilk test. The comparison between mean values (mean RR, SBP, MBP, DBP, CO and PVR) of two successive phases (supine rest vs. HUT and supine recovery vs. MA) was performed using the paired Student's t-test, except

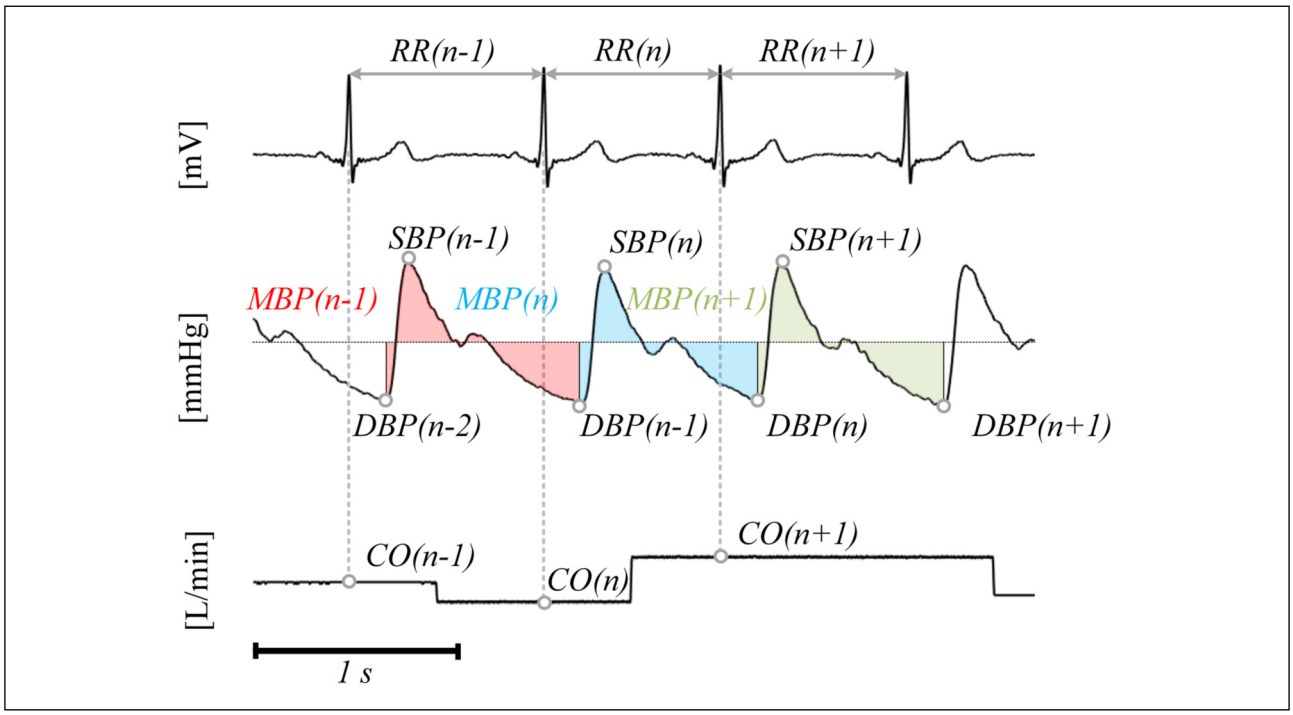

Fig. 2. Conventions for the measurement of RR, SBP, MBP, DBP, and CO variability series; the PVR is calculated as the ratio of *MBP(n)* and *CO(n)*.

comparison of mean values of CO between supine rest vs. HUT phase where, due to the non-normal distribution of the data, the Wilcoxon signed-rank test was used. The statistical comparison among the spectral causality and gain computed along the direction from SBP, MBP, and DBP to RR or PVR was performed using the Friedman test. The comparison of spectral coupling and gain between two phases of the protocol (supine rest vs. HUT and supine recovery vs. MA) was performed by the Wilcoxon signed-rank test. All results were considered statistically significant at a P value <0.05. Statistical analysis was performed using SYSTAT 13 (Systat Software Inc., Chicago, Il, USA).

RESULTS

The basic cardiovascular parameters (mean time series values) averaged among all subjects during the four phases of the protocol are summarized in *Table 1*.

During HUT, RR interval, SBP and MBP, and CO significantly decreased, DBP remained stable, whereas PVR increased. During MA, the length of RR interval was shortened, whereas blood pressure parameters, CO, and PVR significantly increased compared to the previous supine recovery phase.

The distributions of the spectral causal coupling strength along the cardiac chronotropic BR arm evaluated in the LF band using each of the three blood pressure measures as the input signal and RR as the output signal are depicted in the left panel of *Fig. 3*, while the right panel reports the distributions of the spectral causal coupling along the vascular resistance BR arm evaluated in the LF band from each blood pressure measure to PVR.

The analysis of the cardiac chronotropic BR arm showed the dominance of the spectral coupling in the MBP→RR direction. This result was found consistently during the two resting periods and MA, but not during HUT when the spectral causal couplings strength with the RR as the output signal was

Table 1. Basic cardiovascular parameters averaged among all subjects during four phases of the protocol (n=39 for supine rest and HUT phase and n=36 for supine recovery and MA phase).

	Supine rest	**HUT**	**Supine recovery**	**MA**
RR [ms]	906 (92)	726 (82)*	948 (96)	805 (87)*
SBP [mmHg]	124 (9)	118 (9)*	126 (11)	137 (12)*
MBP [mmHg]	92 (6)	90 (6)*	93 (7)	102 (8)*
DBP [mmHg]	72 (6)	73 (6)	73 (6)	80 (7)*
CO [$L.min^{-1}$]	6.61 (1.06)	5.94 (0.93)*	6.65 (1.15)	7.17 (1.41)*
PVR [$mmHg.min.L^{-1}$]	14.3 (2.5)	15.6 (2.5)*	14.4 (2.5)	14.7 (2.7)*

Values are expressed as mean (SD). RR - RR interval, SBP, MBP and DBP - systolic, mean and diastolic blood pressure, respectively, CO - cardiac output, PVR - peripheral vascular resistance. * denotes statistically significant difference between two successive phases (P<0.05, supine rest vs. HUT, supine recovery vs. MA).

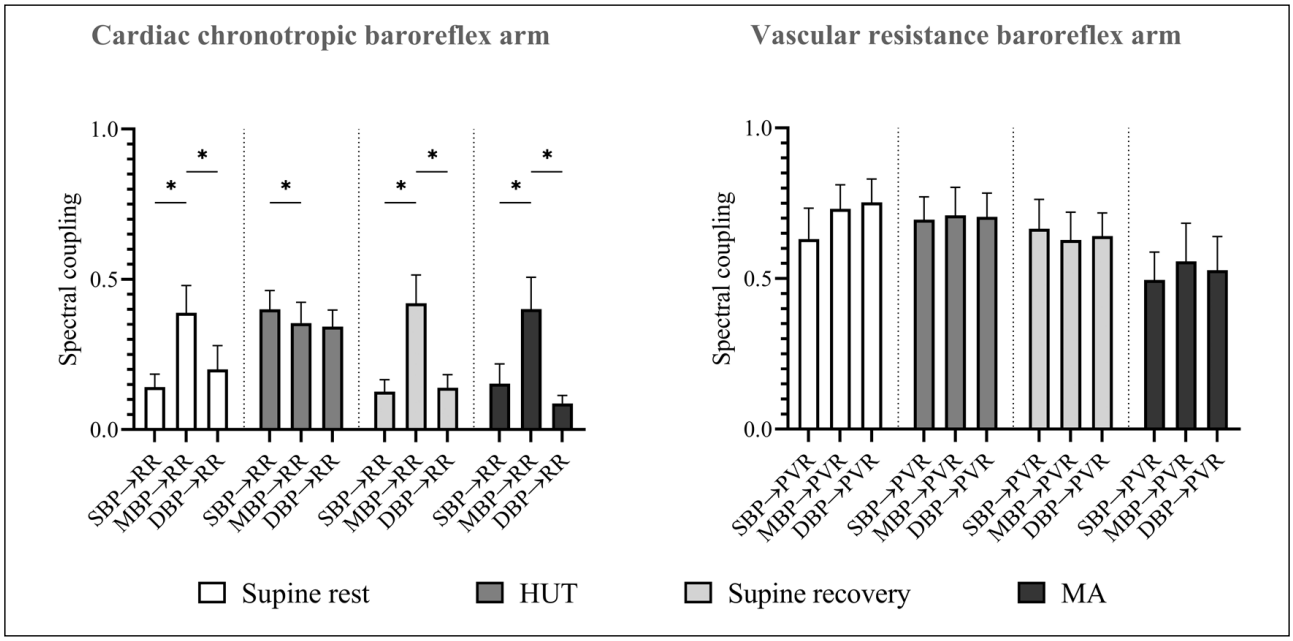

Fig. 3. Spectral causal coupling measured along the direction of interaction from SBP, MBP or DBP to RR (representing cardiac chronotropic baroreflex arm, left panel) and from SBP, MBP and DBP to PVR (representing vascular resistance baroreflex arm, right panel) during the four phases of the protocol (supine rest, HUT, supine recovery, and MA). Distributions are plotted as bars reporting the mean and upper limit of 95% confidence interval. * denotes statistically significant difference (P<0.05) between causal couplings within the same phase.

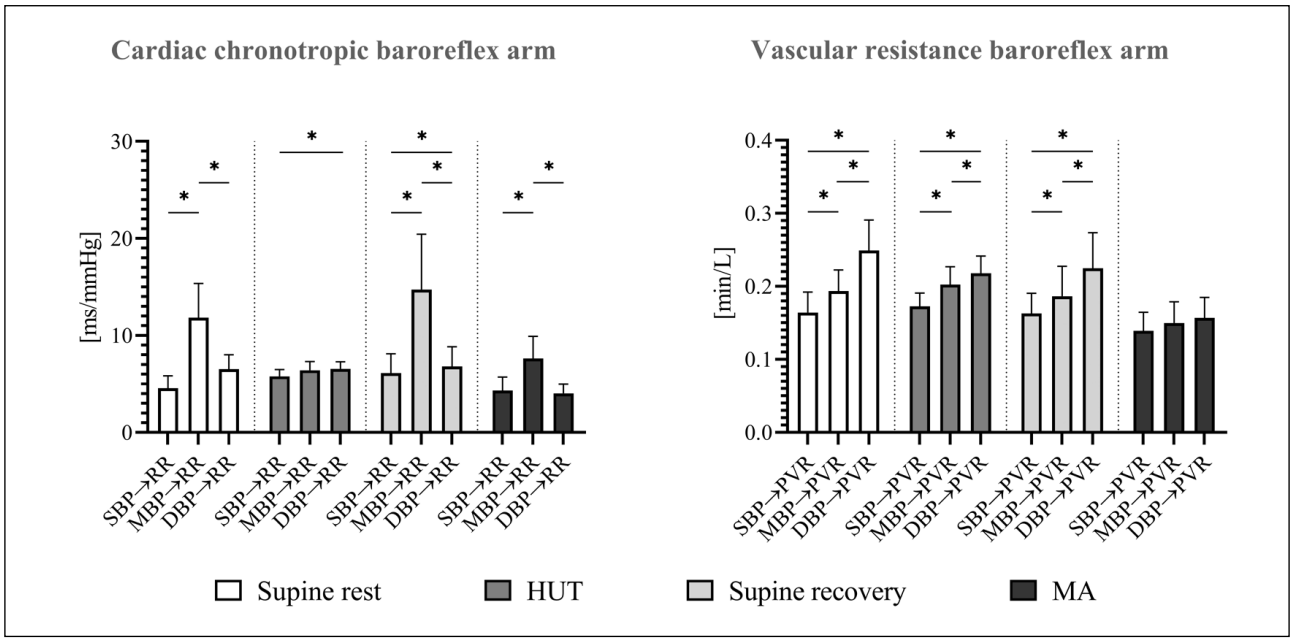

Fig. 4. Spectral gain measured along the direction of interaction from SBP, MBP and DBP to RR (representing cardiac chronotropic baroreflex arm, left panel) and from SBP, MBP and DBP to PVR (representing vascular resistance baroreflex arm, right panel) during the four phases of the protocol (supine rest, HUT, supine recovery, and MA). Distributions are plotted as bars reporting the mean and upper limit of 95% confidence interval. * denotes statistically significant difference (P<0.05) between causal couplings within the same phase.

Table 2. The comparison of spectral coupling and gain between two subsequent phases of the protocol represents the response of these parameters to orthostasis (supine rest vs. HUT) and cognitive challenge (supine recovery vs. MA). P values were obtained by Wilcoxon signed rank test.

	Spectral coupling		**Spectral gain**	
	Supine rest vs. HUT	**Supine recovery vs. MA**	**Supine rest vs. HUT**	**Supine recovery vs. MA**
	P value			
SBP→RR	*< 0.001*	0.447	0.109	0.158
MBP→RR	0.706	0.962	*<0.001*	*< 0.001*
DBP→RR	*< 0.001*	0.087	0.727	*0.007*
SBP→PVR	0.342	*0.008*	0.548	0.078
MBP→PVR	0.718	0.185	0.252	0.060
DBP→PVR	0.483	0.103	0.485	*0.008*

more similar at varying source blood pressure signals with a mild dominance of SBP as an input. As regards the vascular resistance BR arm, no significant differences in spectral coupling from arterial blood pressure signals as the input to PVR as the output signal were found during the whole protocol. In general, the spectral coupling along the vascular resistance BR arm was significantly higher compared to the coupling along the cardiac chronotropic arm.

The results of the analysis of the spectral gain are summarized in *Fig. 4*, where the bar graph in the left and right panels illustrate the gain distributions related to the cardiac chronotropic BR arm and to the vascular resistance BR arm, respectively. In almost all phases of the protocol, the spectral gain of the cardiac chronotropic BR arm was the highest when MBP was taken as input. The spectral gain of the vascular resistance BR arm was highest with DBP as the input in all phases except MA where only a tendency (not reaching statistical significance) to the highest values for the DBP→RR interaction was observed.

The comparison of spectral coupling and gain between two subsequent phases of the protocol representing the response to orthostasis (supine rest vs. HUT) and to cognitive challenge (supine recovery vs. MA) are summarized in *Table 2*.

The spectral coupling along the cardiac chronotropic BR arm increased (P<0.001) as a response to HUT for SBP and DBP as input signals. The spectral gain along this arm significantly decreased in response to both HUT (MBP→RR direction, P<0.001) and MA (MBP→RR direction, P<0.001; DBP→RR direction, P=0.007). Focusing on the vascular resistance BR arm, MA was associated with significantly lower values of spectral causality in the coupling strength from SBP to PVR (P=0.008), and with significantly lower values of spectral gain using DBP as the input signal (P=0.008). In contrast, no significant effect of HUT was

revealed using any of the input blood pressure signals when PVR was considered as an output.

DISCUSSION

Our study was focused on the comparison between the strength of the causal spectral coupling from the input signals SBP, MBP and DBP to the output signals RR (cardiac chronotropic BR arm) or PVR (vascular resistance BR arm), in order to evaluate the most appropriate input signal to be employed in BR analysis. In previous studies, the use of a specific beat-to-beat blood pressure signal for BR analysis was usually justified by functional relevance or correlation analysis (15, 35, 43), but the systematic study that evaluates the strength of the spectral causal coupling in LF band with various blood pressure input signals for BR analysis is still missing.

For the analysis of the cardiac chronotropic BR arm where the heart rate (or its reciprocal, the RR interval) is the output signal, the easily detectable SBP changes were preferably used as the input signal (47). The vascular resistance BR arm was evaluated mainly using the invasive recordings of the MSNA (17, 18, 20, 22, 32), or later with the noninvasive recording of PVR (25, 48), as the output signal, and the DBP as the input signal. Many approaches proposed for the BR function evaluation *via* the analysis of spontaneous fluctuation of cardiovascular parameters are based on an open-loop description of their relationship (do not consider causality), which could potentially lead to misinterpretation of the results in closed-loop system of interactions (49, 50). In order to achieve more objective assessment of the input signal selection, we have analysed the strength of the coupling from the input signal taken from a set of three blood pressure variability series (SBP, MBP, DBP) to the given output signal (RR or PVR) using the partial spectral decomposition method which allows frequency-specific analysis of causal interactions between time series (3, 41, 42).

This approach is more precise compared to the directed coherence analysis and could bring new information about the spectral couplings between parameters characterizing specific BR arm (13, 42, 51). Considering the timescale of PVR and heart rate oscillations associated with the BR function, the analysis was focused on LF oscillations only (37). Our results clearly demonstrate that the strength of coupling depends not only on the analysed baroreflex arm but also on the selection of the input blood pressure signal. During rest phases and cognitive load, the spectral analysis of the cardiac chronotropic BR arm showed a dominant spectral coupling from MBP to RR, while during HUT the importance of SBP as the source signal for the analysis of the cardiac BR arm increased.

The analysis of the vascular resistance BR arm showed similar strength of interconnections between the various blood pressure input signals and PVR. These results contradict previous studies finding that DBP was more closely related to sympathetic nerve activity (MSNA) than SBP (using noncausal analysis in the time (35) or frequency domain (52)).

The possible explanation of the different importance of various blood pressure input signals for the BR response along the cardiac chronotropic arm could be found in previous studies of the high pressure baroreceptors afferent traffic performed on mathematical or animal models (53-56). Baroreceptors, located in the aortic arch wall (aortic sinus) and carotid artery wall (carotid sinus), are mechanically deformed according to blood pressure changes. These deformations lead to a transmission of the impulses to the afferent fibres originating from the carotid sinus *via* the glossopharyngeal nerve and from the aortic sinus *via* the vagal nerve to cardiomotor and vasomotor centres in the brain stem (48, 57-59). An increase in arterial blood pressure evokes a baroreflex response - a decreased sympathetic nerve activity to the heart, arterioles and veins and increased parasympathetic nerve activity to the heart (14, 26). Conversely, a blood pressure decrease is accompanied by the opposite effects. The normal values of arterial blood pressure usually evoke continuous baroreceptor firing during both systolic and diastolic phases, an elevated arterial blood pressure increases the continuous firing even more, and lower values cause bursting mainly during the systolic phase with a more quiescent diastolic phase (60). Therefore, we suggest that the highest importance of SBP as an input signal for baroreflex response obtained during HUT could be attributed to the following mechanism: the relative proportion of systolic phase duration to the whole cardiac cycle increases with an increase in heart rate during orthostasis. Alternatively, since baroreceptors are - in general - less stimulated when blood pressure significantly decreases during HUT, baroreceptors firing during systolic blood pressure peaks could become more

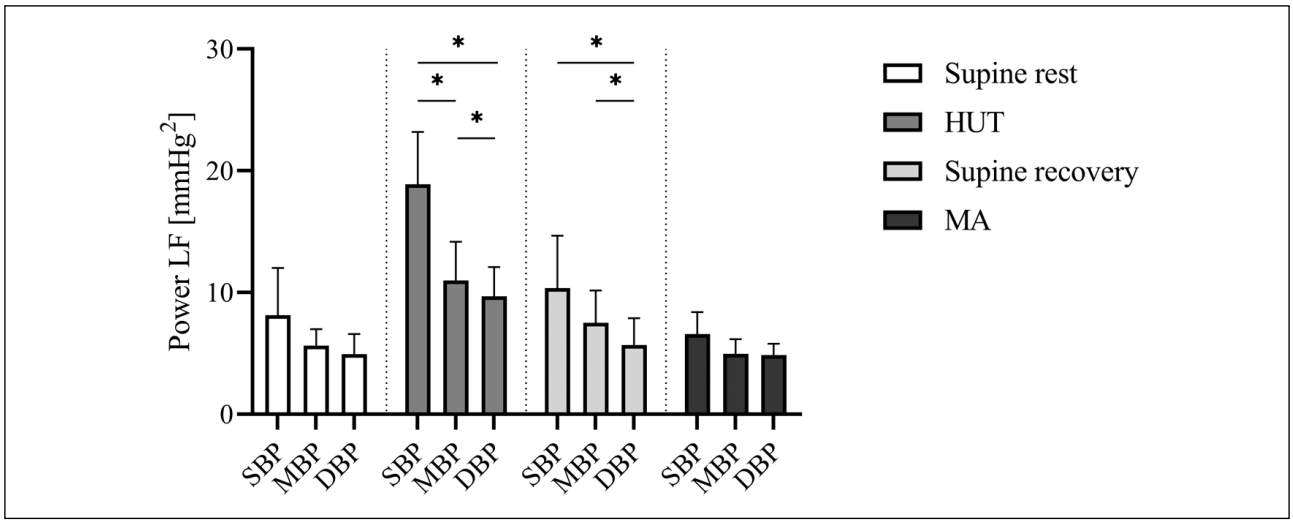

Fig. 5. SBP, MBP and DBP spectral power values in the LF band during four phases of the protocol (supine rest, HUT, supine recovery, and MA). Distributions are plotted as bars reporting the mean and upper limit of 95% confidence interval. * denotes statistically significant difference between spectral power within the same phase ($P<0.05$).

dominant. It could lead to the observed increased coupling between SBP as an input signal compared to other blood pressure signals. However, based on our data we cannot verify the proposed explanations and further studies are needed on this topic. The less prominent shortening of the cardiac cycle together with a significant blood pressure increase during MA did not lead to a similar shift in the baroreflex input signals dominance during this phase. On the other hand, the lack of prevalence of coupling from a specific input signal observed for the vascular resistance BR arm could be attributed to the low-pass filter characteristics of the sympathetic control of vessels diameter. Mathematically, the high mutual correlations existing among the various blood pressure input signals, together with the fact that the output signal is partly determined by MBP (see Eq. 2), explain the tendency of the causal coupling to stabilize to high values regardless of the input signal and the experimental condition (61, 62).

The importance of the given input blood pressure signal (SBP, MBP or DBP) for the BR response was quantified by the spectral coupling strength. In the next step, we have also calculated the gain as a measure of the response magnitude to the unit blood pressure change of cardiac chronotropic or vascular resistance BR arms. The gain is commonly used as a measure of BR sensitivity with its diagnostic and prognostic value (1, 2, 4, 6-9). Here, we generally found that the gain values are not only dependent on the physiological condition, but they are also sensitive to the selected blood pressure input signal. Interestingly, the differences in gain values related to the cardiac chronotropic BR arm were similar compared to those observed for the spectral coupling values, showing the largest gain for the MBP→RR connection. In addition, significant effects of orthostatic and cognitive loads (a decrease in gain associated with vagal withdrawal) were observed only when MBP was taken as the input signal for cardiac chronotropic baroreflex arm analysis. These findings suggest that the MBP should be used preferably over SBP and DBP signals for the analysis of the baroreflex function studied taking the RR intervals as the output. In addition, it was demonstrated that this parameter has also an important prognostic value - in previous studies, the absolute MBP values or its variability, rather than other blood pressure variables, were recognized as a more reliable metric for blood pressure monitoring in an intensive care unit (63-65).

On the other hand, despite the comparable spectral coupling values along the SBP→PVR, MBP→PVR and DBP→PVR directions, gain values observed for the vascular baroreflex arm differed among input signals with the highest values associated with DBP→PVR direction. In this case, since the strength of the coupling directed to PVR is high and stable (*Fig. 3*), finding a higher gain along the direction DBP→PVR than the directions SBP→PVR and MBP→PVR suggests that DBP should exhibit the lowest magnitude of LF oscillations; this was verified comparing the LF power of SBP, MBP and DBP (*Fig. 5*). Also based on these observations, we remark that the gain is a measure of the entity of the reflex rather than of its effectiveness, and confirm that the selection of the input signal should depend on the coupling strength rather than on the gain.

Limitations of this study: Baroreflex control is a complex physiological function characterized by time-variant properties and nonlinear features (49, 66, 67). The frequency domain approach, used in this study, limits the analysis of the reflex to only a small portion of the entire sigmoid reflex arc; thus, the system nonlinearity does not play a significant role, and a linear model is sufficient to accurately describe the observed changes (66, 68, 69). Therefore, given its relative simplicity and the interpretability of the results, the linear model was used in our study.

Previous studies demonstrated hysteresis in the baroreflex function reflected by observed difference in baroreflex mediated responses to increase and decrease in blood pressure. In our study, we did not separately analyse the influence of the rising and falling blood pressure sequences, which can provide more specific information on alterations in autonomic control under physiological and pathophysiological conditions in the context of baroreflex function asymmetry (70). However, the possibility to analyse this phenomenon considering the bidirectional relation between input and output signals in the baroreflex is currently very limited.

Although PVR was mathematically derived using also heart rate as a parameter and thus we cannot exclude the involvement of the cardiac baroreflex arm in this term used for PVR calculation, we suggest that PVR calculated as the ratio including two other degrees of freedom represented by two additionally included independently changing parameters (*i.e.*, MBP and SV) is dominantly dependent on vasomotion rather than on cardiac chronotropic control. Currently we have no other possibility for noninvasive vascular resistance baroreflex arm assessment based on the vasomotion related baroreflex response representing whole systemic circulation.

In conclusions, the most important results related to the aim of the study showed that the strength of the coupling along the baroreflex depends not only on the analysed arm but also on the selection of the source blood pressure signal and the physiological state. At rest and during cognitive load, the MBP signal is the most tightly coupled with RR along the cardiac baroreflex chronotropic arm, while during orthostasis SBP oscillations become more tightly coupled with RR. These results, together with the highest responsiveness of MBP→RR values to changes in physiological state, indicate that MBP should be preferably used for cardiac chronotropic baroreflex analysis. The existence of a preferable blood pressure signal for baroreflex analysis along the vascular resistance arm was not demonstrated.

List of abbreviations: BR, baroreflex; CO, cardiac output; DBP, diastolic blood pressure; ECG, electrocardiogram; HUT, head-up tilt; MA, mental arithmetic task; MBP, mean blood pressure; MSNA, muscle sympathetic nerve activity; PVR, peripheral vascular resistance; SBP, systolic blood pressure; SV, stroke volume

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