Quantification of Cardiac Baroreflex Function at Rest and during Autonomic Stimulation

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Abstract: The cardiac baroreflex constitutes an important mechanism mediating autonomic control of heart activity. Its function can be quantified by applying sequence analysis based on continuous recordings of blood pressure and heart rate. In this study, several indices derived from this method were compared regarding their suitability to estimate baroreflex function at rest and during autonomic stimulation. A cold pressor test was used to induce vagal withdrawal. Changes in the following indices evoked by this procedure were examined: baroreflex sensitivity (the extent of changes in heart period following blood pressure fluctuations, baroreflex effectiveness (the relative frequency in which the reflex responds to blood pressure fluctuations), and baroreflex power (the reflex operations in a defined period). The values of all indices decreased during autonomic stimulation.

The cardiac baroreflex system plays a major role in the short-term regulation of blood pressure. It consists of a negative feedback loop in which activity changes of the arterial baroreceptors resulting from blood pressure fluctuations are responded to by compensatory changes in heart rate [1]. Besides its importance for blood pressure stabilization, this system is involved in the bidirectional interaction between central nervous and cardiovascular processes. Baroreceptor activation exerts a generalized inhibitory effect on the brain [2], including, for example, the reduction of cortical excitability [2], stimulation of sleep [3], and dampening of pain sensitivity [4]. On the other hand, the baroreflex is implicated in central nervous control of the cardiovascular system mediating parasympathetic influences on heart activity [5, 6]. It has been shown, for instance, that pharmacologically induced increases and decreases in vagal outflow are accompanied by activation and inhibition of baroreflex function, respectively, and that tonic parasympathetic cardiac control can be predicted from baroreflex function [7]. Baroreflex function also varies with modulations in autonomic tone associated with different central nervous and psychophysical states. This has, for example, been demonstrated for mental stress [7, 8], cognitive activity [9], and relaxation [8, 10].

Moreover, there is evidence linking aberrances in baroreflex function to cardiovascular disease [11]. It is well-established that the baroreflex is inhibited in essential hypertension [5, 12]. This diminished reflex function is assumed to be involved in the long-term setting of blood pressure and in the etiology of the disease [11–13]. Also, ventricular tachyarrhythmias and coronary artery disease are well-known to be associated with alterations in baroreflex function [14, 15]. Baroreflex control of heart rate furthermore has emerged as a prognostic factor in cardiology [16, 17]. Inhibited baroreflex activity has been shown to predict cardiovascular mortality, particularly sudden cardiac death [16, 18].

Because of the role of the baroreflex as an interface between the central nervous and cardiovascular systems and in consideration of its clinical importance, the quantification of baroreflex function is of high relevance.
end, the spontaneous covariation of systolic blood pressure and heart period is usually analyzed [19]. The application of this technique in the time domain, predominantly referred to as “sequence analysis,” locates cardiac sequences in which the baroreflex reflex is in operation (“baroreflex sequences”). Sequences of consecutive cardiac cycles are sought in which progressive blood pressure increase is accompanied by increase in heart period, or in which blood pressure decrease is accompanied by decrease in heart period [20, 21]. The most established parameter derived from this method is the sensitivity of the baroreflex, which is expressed as the change in heart period per unit change in systolic blood pressure estimated within the baroreflex sequences [8, 21].

A second index describes the effectiveness (also referred to as incidence) of the baroreflex system [22]. The reflex is not always effective in the sense that not all progressive changes in blood pressure are followed by compensatory changes in heart period. The effectiveness index captures the relative portion of systolic blood pressure ramps accompanied by changes in heart period in the expected direction with respect to the total number of blood pressure ramps. The sensitivity and effectiveness of the baroreflex have been postulated not to be redundant, but rather to relate to complementary aspects of baroreflex control on heart activity [9, 22]. Reyes del Paso and colleagues [9], for instance, found differential changes in both indices during mental stress. Dissociations between both features have also been reported during sleep, where baroreflex sensitivity was found to increase and effectiveness to decrease [22].

Finally, the power of the baroreflex is defined as the portion of cardiac cycles that form part of the reflex sequences with respect to the total number of observed cycles. This index is assumed to reflect the relative contribution of the baroreflex to the regulation of cardiac activity [8, 10]. However, in contrast to the other indices, baroreflex power depends on the amount of input to the baroreceptors, i.e., the number of blood pressure ramps [10]. A higher number of blood pressure ramps, for instance, increases the probability of more reflex sequences. Since the index is not adjusted by the number of ramps, it may, to a certain degree, reflect blood pressure variability in addition to properties of the reflex system.

In the present study, the described indices of baroreflex function were compared with respect to their sensitivity to changes in autonomic state. Baroreflex activity has been shown to be closely related to parasympathetic outflow with increasing outflow predicting higher sensitivity [5, 7]. Consequently, reflex sensitivity is expected to decrease in accordance with a reduction in vagal tone, which can be induced by autonomic stimulation. Assuming a general inhibition of the baroreflex in case of vagal withdrawal, we may further hypothesize that during autonomic stimulation, fewer blood pressure ramps are accompanied by changes in heart period in the expected direction (i.e., a reduction in baroreflex effectiveness), and that the number of reflex operations decreases (i.e., a reduction in baroreflex power). A cold pressor test was used to test these predictions. The application of this technique, involving the immersion of a limb in ice-cold water, is known to produce a substantial and reliable decrease in parasympathetic tone [23–25]. Warm-water exposure was additionally used to investigate changes in baroreflex function during less intense stimulation. To control whether the intended autonomic changes were successfully induced, the RMSSD [26] was applied as an index of heart rate variability. It is well known that heart rate variability accurately reflects parasympathetic outflow [27, 28].

Moreover, the indices of baroreflex function were examined with regard to their sensitivity to interindividual differences in tonic blood pressure levels. This criterion was applied because of the comprehensively documented involvement of the baroreflex in the long-term setting of blood pressure [11, 29, 30]. In animals, surgical baroreceptor denervation led to sustained blood pressure elevation [31, 32], whereas prolonged stimulation of the baroreceptor afferents produced sustained blood pressure decrease [33]. Patients with complete denervation of the carotid baroreceptors typically experience severe labile hypertension [34–36], and several studies demonstrated that the baroreflex is inhibited in essential hypertension [5, 12]. Considering this, one may assume that a valid index of baroreflex function is suitable to differentiate between individuals at both ends of the normotensive spectrum.

The reported dissociations between the different features of the baroreflex [9] raise the question of to which degree the described indices reflect common or independent aspects of baroreflex control of heart activity. While baroreflex sensitivity and effectiveness were hypothesized to assess complementary aspects of baroreflex function [9, 22], the role of baroreflex power has not yet been discussed in this regard. Thus, it seemed useful to explore the pattern of interrelations between all three indices. In the current study, the indices of baroreflex function were investigated with respect to their sensitivity to autonomic stimulation and their relationship with tonic blood pressure levels. During the conditions of autonomic stimulation of varying intensities, a valid parameter of baroreflex function is expected to exhibit differentially pronounced reduction. Such a parameter should furthermore differentiate between individuals with varying tonic blood pressure. The pattern of associations between the indices is expected to provide information regarding the degree to which they reflect common or specific functional features of the baroreflex.
METHODS

Participants. Sixty normotensive participants (28 men, 32 women) with systolic blood pressure ranging from 100 to 140 mmHg were included in the study. Exclusion criteria comprised severe physical diseases, psychiatric disorders, and the use of psychoactive drugs or medication affecting the cardiovascular system. All participants were right-handed according to the Edinburgh Handedness Inventory [37]. Fifty of the subjects were university students, 7 were employees, and 3 were self-employed.

The sample was split according to systolic blood pressure into two equally sized groups (systolic median: 119.5 mmHg; gender distribution: 7 men, 23 women in the low blood pressure group; 21 men, 9 women in the high blood pressure group). Information regarding blood pressure and heart rate recorded before the experimental procedure, age, and body mass index (BMI, kg/m²) is presented in Table 1. All participants gave informed consent prior to the experimental session.

Autonomic stimulation and continuous blood pressure recording. The cold pressor test required each subject to immerse his/her left hand and forearm in ice-cold water for 60 s. Water temperature was maintained from 1°C to 3°C. Immediately before immersion, the subjects were exposed to warmth stimulation induced by placing the left hand and forearm in a water bath of 37°C for 3 min.

Blood pressure was measured continuously employing the Penaz method [38] using a FINAPRES 2300 BP Monitor (Ohmeda). Data were recorded by means of a Biopac system (Biopac Systems Inc.). The cuff of the Finapres device was applied to the right index finger of each subject. The hand was positioned at the level of the heart.

Procedure. At the beginning of the experimental session, three sphygmonanometric blood pressure measurements were taken in a sitting position. Readings were separated by 5 min rest intervals. Average systolic values were used to assign subjects to the blood pressure groups. They were requested not to drink alcohol or beverages containing caffeine for three hours prior to experimental sessions.

To determine baroreceptor function, continuous blood pressure measurement was carried out for the duration of four time periods: (i) a baseline phase prior to autonomic stimulation (3 min); (ii) warm-water exposure (3 min); (iii) ice-water exposure (1 min); and (iv) a recovery phase following autonomic stimulation (3 min).

DATA ANALYSIS

Hemodynamic measures. Baroreflex function was analyzed using a software program developed by Reyes del Paso [21]. The program locates sequences of three to six consecutive heart cycles in which systolic blood pressure increases are accompanied by increases in heart period (up sequences), and those in which blood pressure decreases are accompanied by decreases in heart period (down sequences). Since a time lag of one heart beat is known to produce the best estimates of baroreceptor reflex function [39], each systolic value was paired with heart period from the immediate following cycle. One mmHg and 2 ms were applied as minimal criteria for changes in blood pressure and heart period (defined as the intersystolic interval), respectively.

When one of these reflex sequences was detected, the regression line was computed across all cardiac cycles of the given sequence. Baroreceptor sensitivity was expressed as the change in heart period (in ms) per mmHg blood pressure change, measured by the slope of the regression line. The proportion (in %) of systolic blood pressure ramp changes accompanied by corresponding changes in heart rate (i.e., the detected reflex sequences) with respect to the total number of systolic blood pressure ramps (followed or not followed by reflex changes in heart period) was applied as an indicator of baroreflex effectiveness. Baroreflex power was defined as the percentage of cardiac cycles forming part of the detected reflex sequences with respect to the total number of cycles during the analyzed period. All parameters were obtained differentially for the up and down, as well as for the detected reflex sequences in their entirety.

Heart period and heart period variability (RMSSD) [26] were additionally observed. To gain a better insight into the role of input to the baroceptors for the indices of reflex function, we also included the number of blood pressure ramps in the analysis. All parameters were com-

<table>
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<tr>
<th>Table 1.</th>
<th>Systolic blood pressure, diastolic blood pressure, age, and BMI in both blood pressure groups: means (M), standard deviations (SD), minimal (Min), and maximal (Max) values.</th>
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<td></td>
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<td>BMI (kg/m²)</td>
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puted separately for the four experimental conditions (baseline, warm-water exposure, ice-water exposure, recovery).

**Statistical analysis.** Changes in baroreflex function over the experimental conditions were evaluated using ANOVA procedures, with the four conditions as within subjects factor, and blood pressure group (high vs. low blood pressure) as between subjects factor. The dependent variables comprised the indices of baroreflex sensitivity, effectiveness and power, as well as heart period and RMSSD. The number of systolic blood pressure ramps (both progressive upward and downward changes, accompanied and not accompanied by corresponding changes in heart period) was compared between the blood pressure groups using a MANOVA procedure. Interrelations between the parameters of baroreflex function were determined by computing Pearson correlations between sensitivity, effectiveness, and power indices derived from the baseline measurement across the total sample.

**RESULTS**

Figures 1 to 3 display the values of the sensitivity, effectiveness, and power indices for the four conditions. The results of the ANOVA procedures are provided in Table 2.

A slight decrease with respect to baseline was observed for baroreflex sensitivity during warm-water exposure, followed by a stronger reduction during ice-water stimulation. During the recovery period, sensitivity increased and almost reached baseline level. This held true for the up and down sequences, as well as across all detected reflex sequences. Similar patterns were observed for the indices of baroreflex effectiveness and power. However, while almost all parameters of effectiveness and power substantially decreased under ice-water stimulation, the decline during warm-water exposure was less consistent than for the measures of baroreflex sensitivity.

Baroreflex sensitivity was higher in the low blood pressure group than in the high blood pressure group. This was the case concerning the reflex sequences in their entirety, as well as for the up and down sequences. An opposite pattern was observed for baroreflex power. The three power indices were higher in the high blood pressure group under each condition. Merely nonsystematic group differences were found for baroreflex effectiveness.

The changes in baroreflex function during autonomic stimulation were confirmed by a significant main effect of the factor condition in the ANOVAs for each of the included variables, excepting the power index for the up sequences (see Table 2). A significant main effect of blood pressure group was found for the sensitivity index derived from the up and total sequences, as well as for the three power indices. A significant interaction effect between condition and blood pressure group was found only for the effectiveness index revealed from the up sequences, apparently resulting from the higher values in the low blood pressure group during baseline.

The modulations in heart period and heart period variability (RMSSD) are presented in Fig. 4. The values of both parameters were lower in the high blood pressure group and decreased during warm-water and more so during ice-water exposure. The effect of condition, but not the group effect, reached significance in both cases (Table 2).
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Figure 5 displays the number of systolic blood pressure ramps (progressive upward and downward changes) per minute in both blood pressure groups. More ramps were found in the high blood pressure group under each condition indicating higher blood pressure variability. This held true for the ramps followed by corresponding changes in heart period (reflex ramps), as well as for those for which this was not so (non-reflex ramps). Multivariate testing (MANOVA) revealed a significant effect of blood pressure group on these variables ($F[16/43] = 2.07, P = 0.030$).

Table 3 provides the correlation matrix for the indices of baroreflex function derived from the baseline measurement calculated across the entire sample. Significant positive associations were found between most of the parameters. The only exception comprised interrelations between the three indices of baroreflex sensitivity and those of power, with all respective correlations being close to zero.
The cardiac baroreflex system is one of the most relevant physiological mechanisms mediating cardiovascular autonomic control with its functional properties being strongly influenced by the parasympathetic system [5–7, 11]. This was documented, for instance, by Reyes del Paso and colleagues [7] who found that both pharmacologically induced inhibition and augmentation of vagal activity are accompanied by corresponding changes in the sensitivity of the reflex. The same study revealed a substantial correlation of baroreflex sensitivity with respiratory sinus arrhythmia, which constitutes a widely accepted measure of parasympathetic tone [40–42].

The present study revealed significant changes during autonomic stimulation in the employed indices of baroreflex function. Reflex sensitivity decreased slightly during warm water exposure and more so during the cold pressor test. During recovery, it once again increased and almost reached baseline level. Given the relationship between baroreflex function and vagal tone, the observed changes can be interpreted as reflecting different degrees of parasympathetic withdrawal due to autonomic stimulation. The effectiveness of the baroreflex was also modulated by the applied stimulation procedure. However, the pattern of change proved less consistent. While effectiveness substantially decreased during ice-water stimulation, no consistent reduction was observed during warm-water exposure. A similar result was obtained for baroreflex power. However, only the power indices for the up and the total reflex sequences decreased in the ice-water condition. Again, only non-systematic changes emerged during warm water exposure. In light of these findings, baroreflex sensitivity would appear to be most sensitive to modulations in autonomic tone, particularly to those of lesser intensity.

The RMSSD was applied as a manipulation check regarding the efficiency of the stimulation procedure. It is a valid indicator of vagal tone that is highly correlated with
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**Respiratory Sinus Arrhythmia** [26]. Therefore, the slight decrease of the RMSSD during warm-water exposure and its stronger decrease during cold pressor stimulation underline that parasympathetic withdrawal of different magnitudes was successfully induced. It should, however, not be overlooked that besides vagal withdrawal, ice-water stimulation triggers activation of the sympathetic system, which is accompanied by heart acceleration and increasing vascular resistance [43, 44]. Thus, the significant decline in heart period during the experimental procedure...
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may have been mediated by both the sympathetic and the parasympathetic systems.

Furthermore, possible implications of sympathetic activation on the baroreflex system may be discussed. Sympathetically transmitted blood pressure rise implicates heightened input on the baroreceptors, as well as a compensatory reflex response. The degree of compensation, and thus the magnitude and duration of the blood pressure response depend on the functional characteristics of the baroreflex system. Also changes in these parameters resulting from sympathetic activation may be taken into account. Given that the reflex mainly underlies parasympathetic control, no significant direct sympathetic effects on the indices under study may be assumed. However, sympathetically mediated cardiovascular changes may have a secondary impact on baroreflex function. One may, for instance, speculate that the sympathetic reaction also includes increasing blood pressure variability, and thus a higher number of blood pressure ramps. In turn, this could produce a distortion of the power index, which is not controlled for blood pressure variability. Also, distortions because of cardiovascular modulations caused by respiratory changes during cold pressure stimulation cannot be ruled out.

The comparison between the two sub-samples composed according to blood pressure revealed less pronounced baroreflex sensitivity in individuals at the higher end of the normotensive range. This finding is in line with the well-established involvement of the baroreflex in the long-term setting of blood pressure [11, 29, 30] and inhibition of the baroreflex in chronic hypertension [5, 12]. It is also consistent with the assumption of an inverse relationship between parasympathetic tone and resting blood pressure, which is, for instance, expressed in the association between blood pressure and respiratory sinus arrhythmia [13, 45, 46]. Significant differences between both blood pressure groups, however, were observed only for the sensitivity index derived from up sequences. This is consistent with previous findings suggesting that baroreceptor reflex sensitivity more accurately reflects parasympathetic cardiac control during periods of blood pressure increase compared with blood pressure decrease [7, 10]. Baroreceptor effectiveness, which did not differ between the two blood pressure groups, evidently does not reflect the contribution of the baroreflex to the long-term regulation of blood pressure as accurately as the sensitivity index.

Interestingly, the power of the baroreflex was related to blood pressure in a direction opposite to its sensitivity, with significantly higher power values obtained in the high blood pressure group. This may be explained by taking into account the dependence of power on the input to the baroreceptor system [10]. In the present study, significantly more blood pressure ramps (reflex and non-reflex ramps) were found in the sub-sample with higher blood pressure. This implies stronger input to the baroreceptors in terms of a higher rate of stimulation. Therefore, one may hypothesize that besides properties of the baroreflex, the power index reflects the degree of blood pressure variability. Given this apparent confounding of power and blood pressure variability, the suitability of this indicator for the assessment of interindividual differences in baroreflex function would appear to be restricted.

Correlation analysis displayed significant interrelations between most indices of baroreflex function. The highest correlations were found between the values derived from the up sequences and those derived from the down sequences concerning the very same parameter of baroreflex function. This held true for sensitivity, effectiveness, and power. Thus in many questions of research, little information may be lost by restricting the analysis to the total of the reflex sequences. However, given the closer relationship of the sensitivity index derived from the up sequences with parasympathetic tone and the superior sensitivity of this index to some psychological manipulations such as mental stress or relaxation [7, 10], its specific use may also prove appropriate in certain cases.

Midrange correlations were found between the indices of sensitivity and effectiveness suggesting that they reflect similar, though certainly not identical aspects of baroreflex function. This is in accordance with dissociations between sensitivity and effectiveness of the baroreflex system, which have been reported to occur in cognitive-attentional processes as well as during sleep [9, 22]. The power indices were correlated with those of effectiveness, but not with those of sensitivity. Effectiveness and power assess baroreflex function in a somewhat similar manner. Effectiveness relates to the number of operations of the baroreflex over time, i.e., the number of reflex sequences, and power to the heart cycles included in these sequences. Thus, both indices quantify related features of baroreflex function. The sensitivity and power indices quantify reflex function based on rather different approaches. Sensitivity measures the extent of changes in heart activity following blood pressure fluctuations indexed by the slope of the regression line. In contrast, power refers to the number of heartbeats within baroreflex sequences as a portion of the analyzed cardiac cycles. So it is not surprising that these parameters are virtually unrelated.

One limitation of all parameters derived from sequence analysis is that they exclusively take into account reflex changes in heart period. It is assumed that besides heart period, the efferent branch of the baroreflex modulates cardiac contractility [40]. Thus baroreflex function could be quantified even more precisely, also taking into account compensatory changes in stroke volume or cardiac output. These parameters can be relatively easily derived from impedance cardiography or continuous blood pressure recording [40, 47].
In summing up, the study provides evidence for the suitability of the indices derived from sequence analysis in detecting changes in baroreflex function associated with modulations in the autonomic state. Altogether, the most consistent results were obtained for baroreceptor sensitivity, which justifies its common use as a central parameter of baroreflex function [7, 8]. This index also reflects complementary aspects of reflex function, even though they are evidently less reliable. Effectiveness would appear to be superior to power, since it is adjusted according to input to the baroreceptors and is therefore not confounded by blood pressure variability.

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