Ability of short-time Fourier transform method to detect transient changes in vagal effects on hearts: a pharmacological blocking study

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AUTONOMIC NERVOUS SYSTEM (ANS) plays an important role in homeostatic regulation. It is responsible for adaptive changes in heart rate and blood pressure, reacting to intrinsic and extrinsic challenges (21). During the last two decades, spectral analysis of heart rate variability (HRV) has been extensively used to assess ANS activity. The spectrum of HRV signal reveals three major components of spectral power: very low-frequency power (VLFP, <0.04 Hz), low-frequency power (LFP, 0.04–0.15 Hz), and high-frequency power (HFP, 0.15–0.40 Hz) (36). Blockades and other invasive provocations have established that HFP reflects phasic variations in vagal effects on the heart within a respiratory cycle (2, 3, 12, 23, 31, 33). Mechanisms responsible for VLFP and LFP are not fully understood, but it is suggested that both the vagal and sympathetic branches of ANS affect VLFP and LFP (2, 31, 33).

Conventional spectral analysis methods of HRV, such as fast Fourier transform and autoregressive modeling, require the processed signal to be stationary in the analyzed temporal window (36). Therefore, these methods are restricted to steady-state conditions, such as rest in a supine posture. Although calculations of HRV during steady-state conditions provide important information regarding individual subjects or a special physiological condition, they only reflect a limited aspect of ANS regulation (1). Recently, several review articles have clearly stated the importance of assessing dynamic changes in ANS activity, particularly the transient changes in vagal activity, and introduced new analyzing methods that allow us to calculate HRV also from transiently changing (i.e., nonstationary) signals (1, 14, 27). The time frequency or time-variant methods, such as short-time Fourier transform (STFT), selective discrete Fourier transform algorithm (SDA), Wigner-Ville distribution, and time-variant autoregressive modeling, provide estimates of spectral power as a function of time (1, 14, 16, 25, 27).

Traditional approaches to studying the validity of noninvasive indexes of ANS activity utilize pharmacological blockades and/or physiological tests known to provoke changes in ANS activity (2, 6, 24, 25, 27). The pharmacological blockades of either cholinergic or adrenergic innervations serve as an invasive tool for eliminating vagal and sympathetic effects on the heart, respectively (6). The residual level of a particular noninvasive ANS index, such as HFP or R-R interval (RRI), after the blockade of a single branch of ANS provides an estimate of the effects of the unblocked branch on that index (6). Similarly, the response obtained under the selective blockade of a single autonomic branch provides an estimate of the phasic response of the unblocked branch (6).

Active orthostatic task (AOT) is a simple noninvasive autonomic test provoking well-documented changes in autonomic cardiovascular regulation (17, 18, 37). An active change of posture from sitting (or supine) to standing causes a shift of blood away from the chest to the distensible venous capacitance system of the lower limbs (37). To maintain an adequate level of arterial blood pressure for the perfusion of vital organs, especially the brain, requires compensatory adjustments in the cardiovascular system (37). The rapid short-term adjustments are primarily mediated by the neural regulatory system, and the humoral regulatory system only becomes involved during prolonged standing (37). The cardiac response to cholinergic...
innervations is fast, with little delay, whereas the response to adrenergic innervations is slower, with a time delay of 1–2 s (5). Thus the immediate shortening of the R-R interval length after the onset of the standing posture results primarily from vagal withdrawal and secondarily from sympathetic activation (18, 37). Sympathetic activation also increases vasoconstriction in skeletal muscles, kidneys, and the splanchnic bed (37).

The aim of the present study was to evaluate the capability of the STFT method (30), the most conventional method for time-frequency representation, to track the transient changes in vagal effects on the heart. The STFT method has previously been introduced in simulation experiments (16) and applied in the analysis of several biological signals, including HRV data during sleep (16) and dynamic exercise (32). We derived HFP with the STFT method as a function of time during AOT and calculated the HFP reactivity scores describing the initial fast and slow response to AOT. We used selective pharmacological autonomic blockades to separate the vagal and sympathetic effects on the heart during AOT.

MATERIALS AND METHODS

Subjects. Eleven healthy male volunteers [means (SD): age, 23.5 (2.3) yr; height, 181.2 (5.3) cm; weight, 77.0 (9.2) kg; body fat 15.7 (4.5)%] were studied. Seven subjects from the original 18 subjects were excluded from the present results because only those subjects whose respiration was within 0.20–0.40 Hz were selected. The general health status of the subjects was assessed with a brief health questionnaire and a resting ECG. The health questionnaire screened the inherited propensity to cardiovascular diseases, autonomic nervous system abnormalities, and contraindications to vagal blocking. All subjects were medication free. They were asked to maintain their regular lifestyle and refrain from extra physical exertion starting 3 days before the test sessions. During these days, the consumption of alcohol and caffeinated beverages was prohibited. All subjects were nonsmokers. The subjects gave written informed consent to partici- pate, and they had the right to withdraw from the study at any time. The study was approved by the Ethics Committee of the Central Hospital of Central Finland.

Protocol. Subjects were tested on two separate days, including either vagal or sympathetic blockade. The order of autonomic blockades was counterbalanced across days and subjects. In the morning of the test days, the subjects entered a quiet laboratory room (23–24°C) 2 h after breakfast, at 8:15 AM. After catheter insertion into the antecubital vein, instrumentation, and instructions, the subjects rested with the STFT method as a function of time during AOT and calculated the HFP reactivity scores describing the initial fast and slow response to AOT. AOT was carried out in a drug-free condition as a baseline and slow response to AOT. We used selective pharmacological autonomic blockades to separate the vagal and sympathetic effects on the heart during AOT.

The present interest was to obtain information about time-depen- dent changes in HFP (0.20–0.40 Hz). HFP is mediated almost entirely by the vagal nervous system and provides a selective, noninvasive measure of vagal effects on the heart (2, 3, 11, 12, 23, 31, 33). To obtain a sufficiently high time resolution, we chose 0.20 Hz as the lower bound of the high-frequency band, ranging between 0.15 Hz used in most studies. The frequency and time resolutions of the STFT method are inversely related, and therefore, a compromise is always required between these resolutions. It is recommended that the dura- tion of the time window should be at least five times the slowest analyzed wavelength (4, 15, 25). The 25-s time window used in the present study was five times the duration of the lowest variations in the high-frequency band, ranging between 0.20 and 0.40 Hz. HFP was obtained as a function of time by calculating integrals of the power spectral density curve within the above-mentioned frequency band.

HRV analysis. Signal processing and HRV calculations were performed by using the MATLAB program (Program on MATLAB 7, The MathWorks, 2004). The RRI series were checked and edited for artifacts by using a detecting algorithm and subsequently verified by visual inspection. The original RRI series were resampled at a rate of 5 Hz by using linear interpolation to obtain equidistantly sampled time series. A 500-order polynomial filter was used to remove low-frequency trends from the RRI time series. The data were further filtered and detrended by a digital FIR band-pass filter to remove variances below 0.04 Hz and above 1.0 Hz (see e.g., Refs. 30, 34, and 35).

To quantify vagal regulation during steady-state conditions as well as during dynamic changes, the STFT method, an extension of the Fourier transform, was chosen for HRV analysis (see e.g., Ref. 30). The method provided a time-frequency decomposition of the RRI time series by calculating consecutive power spectra of short sections of the signal. A section of 125 samples was multiplied by the window function (a 25-s time window, Hanning, and the fast Fourier transform of their product was taken. The window was then shifted one sample ahead, and the same calculations were performed again. This process was repeated until the whole RRI time series, including sitting for 5 min and standing for 3 min, was covered. The time-frequency distribution can be displayed as a three-dimensional graph, as shown in Fig. 1. Such time-frequency distribution allowed close monitoring of any changes in power (amplitude) or frequency at any given time.

The present interest was to obtain information about time-de pendent changes in HFP (0.20–0.40 Hz). HFP is mediated almost entirely by the vagal nervous system and provides a selective, noninvasive measure of vagal effects on the heart (2, 3, 11, 12, 23, 31, 33). To obtain a sufficiently high time resolution, we chose 0.20 Hz as the lower bound of the high-frequency band, ranging between 0.15 Hz used in most studies. The frequency and time resolutions of the STFT method are inversely related, and therefore, a compromise is always required between these resolutions. It is recommended that the dura- tion of the time window should be at least five times the slowest analyzed wavelength (4, 15, 25). The 25-s time window used in the present study was five times the duration of the lowest variations in the high-frequency band, ranging between 0.20 and 0.40 Hz. HFP was obtained as a function of time by calculating integrals of the power spectral density curve within the above-mentioned frequency band.

RRI, HFP, SBP, and DBP at different steps of AOT. RRI, HFP, SBP, and DBP values were calculated for three different steps of AOT: J) mean from 150 to 270 s in the baseline sitting posture (RRIbg,
HFPbl, SBPbl, and DBPbl); 2) minimum value during the first 30 s after active standing up (RRImin, SBPmin, and DBPmin) and a local minimum HFP value between standing up and RRImin (HFPmin); and 3) mean from 60 to 160 s in the standing posture (RRIstand, HFPstand, SBPstand, and DBPstand).

Reactivity scores describing the initial fast (sitting baseline / HFPmin) and slow (sitting baseline / HFPstand) autonomic cardiac response to AOT were calculated for HFP, RRI, and heart rate (HR):

\[ \text{HFP}_{\text{fast}} = \frac{\text{HFP}_{bl}}{\text{HFP}_{min}}; \quad \text{HFP}_{\text{slow}} = \frac{\text{HFP}_{bl}}{\text{HFP}_{stand}}; \]

\[ \text{RRIfast} = \frac{\text{RRI}_{bl}}{\text{RRI}_{min}}; \quad \text{RRIfast} = \frac{\text{RRI}_{bl}}{\text{RRI}_{stand}}; \]

\[ \text{HRfast} = \frac{\text{HR}_{bl}}{\text{HR}_{max}}; \quad \text{HR}_{\text{slow}} = \frac{\text{HR}_{bl}}{\text{HR}_{stand}}. \]

In addition to RRI, the reactivity change scores were calculated for HR because in some instances the choice of RRI versus HR as a metric for cardiac chronotropy alters the interpretation of autonomic regulation underlying the cardiac response (7, 9).

Respiratory frequency during AOT. The mean respiratory frequency (RF) during AOT was calculated from respiratory intervals. We did not control the RF or tidal volume. Spontaneous RF, and consequently also HFP, may be distributed over a much wider range than the frequency range used in the present study. Thus HFP calculated within 0.20–0.40 Hz might not include all the variations in R-R intervals within a respiratory cycle. For these reasons, only 11 subjects with their RF ranging between 0.20 and 0.40 Hz were included in the present results.

Statistical analysis. To meet the assumptions of parametric statistical analysis, a natural log transformation of the values of HFP was used. HFP, RRI, HR, SBP, and DBP were evaluated for effects due to the blockade and due to the different steps of AOT by 2 (Drug Condition) × 3 (AOT Step) repeated measures ANOVA. Similarly, HFP, RRI, and HR reactivity scores were evaluated for effects due to the blockade and due to the time from the onset of standing posture by 2 (Drug Condition) × 3 (Response Time) repeated measures ANOVA. Differences in RF were compared with a paired sample t-test. Differences between means were considered significant when \( P < 0.05 \). \( \alpha \)-Level adjustments were made as needed to account for numerous pairwise comparisons. All values are means (SD).

RESULTS

Time courses of RRI and HFP responses to AOT. The time courses of the drug-free transient changes in RRI and HFP...
during the first 30 s after standing up were similar on both test days. Before the vagal blockade, $\text{RRI}_{\text{min}}$ occurred 17 s (SD 7) and $\text{HFP}_{\text{min}}$ occurred 13 s (SD 6) after standing up, and before the sympathetic blockade, the corresponding times were 15 s (SD 3) and 10 s (SD 4). Neither vagal [RRI$_{\text{min}}$ 20 s (SD 2) and HFP$_{\text{min}}$ 17 s (SD 4)] nor sympathetic blockade [RRI$_{\text{min}}$ 13 s (SD 3) and HFP$_{\text{min}}$ 11 s (SD 3)] had a significant effect on the time course of transient changes in RRI and HFP during the first 30 s after standing up.

**RRI and HFP at different steps of AOT.** Before the blockade, none of the RRI or HFP values differed significantly between the test days. Figure 2 shows RRI and HFP at the different steps of AOT before the blockade, after vagal blockade, and after sympathetic blockade.

The effects of the vagal blockade on RRI and HFP were evaluated by 2 (Drug Condition) $\times$ 3 (AOT Step) repeated measures ANOVA. Significant main effects for the Drug Condition and AOT Step were found for RRI and HFP ($P < 0.001$). Significant Drug Condition $\times$ AOT Step interactions were found for RRI and HFP ($P < 0.001$). Pairwise comparisons showed that the vagal blockade decreased the RRI and HFP values at all steps of AOT ($P < 0.001$). Pairwise comparisons before the vagal blockade showed that RRI$_{\text{min}}$ and RRI$_{\text{stand}}$ were significantly lower than RRI$_{\text{bl}}$ ($P < 0.001$), and that RRI$_{\text{stand}}$ was significantly higher than RRI$_{\text{min}}$ ($P < 0.01$). Similarly, HFP$_{\text{min}}$ and HFP$_{\text{stand}}$ were significantly lower than HFP$_{\text{bl}}$ ($P < 0.001$), and HFP$_{\text{stand}}$ was significantly higher than HFP$_{\text{min}}$ ($P < 0.001$) before the vagal blockade. Pairwise comparisons after the vagal blockade showed that RRI$_{\text{min}}$ and RRI$_{\text{stand}}$ were significantly lower than RRI$_{\text{bl}}$ ($P < 0.001$), and RRI$_{\text{stand}}$ was significantly higher than RRI$_{\text{min}}$ ($P < 0.05$). In contrast, the HFP values did not differ significantly between the different steps of AOT after the vagal blockade.

The effects of the sympathetic blockade on RRI and HFP were evaluated by 2 (Drug Condition) $\times$ 3 (AOT Step) repeated measures ANOVA. Significant main effects for the Drug Condition were found for RRI ($P < 0.001$) and HFP ($P < 0.01$). Similarly, significant main effects for the AOT Step were found for both variables ($P < 0.001$). The Drug Condition $\times$ AOT Step interaction was significant for RRI ($P < 0.05$) but not for HFP. Pairwise comparisons revealed that the sympathetic blockade increased RRI during all steps of AOT ($P < 0.001$), HFP$_{\text{min}}$, and HFP$_{\text{stand}}$ ($P < 0.01$), but not HFP$_{\text{bl}}$ (see Fig. 2). Pairwise comparisons within the Drug Condition, before and after the sympathetic blockade, showed a similar pattern of significant differences between the different steps of AOT: RRI$_{\text{min}}$ and RRI$_{\text{stand}}$ were significantly lower than RRI$_{\text{bl}}$ ($P < 0.001$), and that RRI$_{\text{stand}}$ was significantly higher than RRI$_{\text{min}}$ ($P < 0.01$). Similarly, HFP$_{\text{min}}$ and HFP$_{\text{stand}}$ were significantly lower than HFP$_{\text{bl}}$ ($P < 0.001$), and HFP$_{\text{stand}}$ was significantly higher than HFP$_{\text{min}}$ ($P < 0.001$) before the sympathetic blockade. Pairwise comparisons after the sympathetic blockade showed that RRI$_{\text{min}}$ and RRI$_{\text{stand}}$ were significantly lower than RRI$_{\text{bl}}$ ($P < 0.001$), and RRI$_{\text{stand}}$ was significantly higher than RRI$_{\text{min}}$ ($P < 0.05$). In contrast, the HFP values did not differ significantly between the different steps of AOT after the sympathetic blockade.

![Fig. 2. RRI, heart rate (HR), and HFP at different steps of AOT before and after selective autonomic blockades. Results from 2 (Drug Condition) $\times$ 3 (AOT Step) repeated measures ANOVAs are presented. For sake of clarity, results from pairwise comparisons are only presented in text. Abbreviations for the steps of AOT: RRI$_{\text{bl}}$, HR$_{\text{bl}}$, and HFP$_{\text{bl}}$, sitting baseline; RRI$_{\text{min}}$, HR$_{\text{min}}$, and HFP$_{\text{min}}$, minimum during first 30 s after active standing up (see MATERIALS AND METHODS); RRI$_{\text{stand}}$, HR$_{\text{stand}}$, and HFP$_{\text{stand}}$, standing posture.](http://ajpheart.physiology.org/)}
significantly lower than HFP_{bl} (P < 0.001), and HFP_{stand} was significantly higher than HFP_{min} (P < 0.001).

Reactivity scores. The reactivity scores describing the fast and slow autonomic cardiac response to AOT were similar before the vagal and sympathetic blockades. The reactivity scores before the blockade, after vagal blockade, and after sympathetic blockade are shown in Fig. 3.

The effects of the vagal blockade on the reactivity scores were evaluated by 2 (Drug Condition) × 2 (Response Time) repeated measures ANOVA. Significant main effects for the Drug Condition and Response Time were found for the RRI, HR, and HFP reactivity scores (P < 0.05–0.001). Also, significant Drug Condition × Response Time interactions were found for the RRI (P < 0.001), HR (P < 0.001), and HFP (P < 0.05) reactivity scores. Pairwise comparisons showed that the vagal blockade affected all reactivity scores significantly (P < 0.05–0.001), except for HR_{slow}. Differences between the fast and slow reactivity scores were significant for all pairwise comparisons (P < 0.05–0.001).

The effects of the sympathetic blockade on the RRI, HR, and HFP reactivity scores were evaluated by 2 (Drug Condition) × 2 (Response Time) repeated measures ANOVA. A significant main effect for the Drug Condition was found for the HR (P < 0.001) but not RRI and HFP reactivity scores. Significant main effects for the Response Time were found for the RRI (P < 0.001), HR (P < 0.001), and HFP (P < 0.01) reactivity scores. Significant Drug Condition × Response Time interactions were found for the RRI (P < 0.001) and HR (P < 0.001) reactivity scores but not for the HFP reactivity scores. Pairwise comparisons showed that the sympathetic blockade affected only ΔHR_{fast} and ΔHR_{slow} significantly (P < 0.001). Differences between the fast and slow reactivity scores were significant for all pairwise comparisons (P < 0.05–0.001).

Time courses of SBP and DBP responses to AOT. The time courses of the drug-free transient changes in SBP and DBP during the 30 s after standing up were similar on both testing days. The time delay between the standing up and occurrence of SBP_{min} and DBP_{min} were similar before and after the vagal blockade [SBP_{min} 12 s (SD 2) vs. 12 s (SD 2); DBP_{min} 12 s (SD 2) vs. 12 s (SD 3), respectively] and before and after the sympathetic blockade [SBP_{min} 12 s (SD 3) vs. 12 s (SD 1); DBP_{min} 11 s (SD 2) vs. 11 s (SD 2), respectively].

SBP and DBP at different steps of AOT. Before the blockade, none of the SBP or DBP values differed significantly between the test days. The effects of the blockades on SBP and DBP were evaluated by similar 2 (Drug Condition) × 3 (AOT Step) repeated measures ANOVAs as computed for RRI and HFP. Both vagal and sympathetic blockades showed significant Drug Condition × AOT Step interactions for SBP and
Vagal effects on the heart. The present blocking experiment confirmed that the fast vagal effects on the heart during AOT. In the drug-free condition, HFP showed a gradual increase to the level characteristic for the standing posture: Standing up results in a transient fall in arterial blood pressure, which is compensated by reflexive vagal inhibition and sympathetic activation (4, 18, 37). Because of the neural regulatory compensation, arterial blood pressure increases rapidly, causing rapid vagal activation and finally, stabilization of the heart rate. The present results further showed that the magnitude of both HFP reactivity scores, i.e., the new fast and conventional slow HFP response to AOT, was abolished by the vagal blockade and unaffected by the sympathetic blockade (see Fig. 3). These findings suggest that HFP derived from the STFT method was able to detect and quantify a decrease in vagal activity during the unstable phase of AOT, where sudden changes occurred in arterial blood pressure and RRI length. The findings are in line with the presumed mechanism of the immediate response elicited by assuming a standing posture: Standing up results in a transient fall in arterial blood pressure, which is compensated by reflexive vagal inhibition and sympathetic activation (4, 18, 37). Because of the neural regulatory compensation, arterial blood pressure increases rapidly, causing rapid vagal activation and finally, stabilization of

Table 1. Blood pressure at the different steps of AOT before and after vagal blockade

<table>
<thead>
<tr>
<th></th>
<th>No Drug</th>
<th>Atropine</th>
<th>Drug Condition</th>
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<tbody>
<tr>
<td><strong>SBP, mmHg</strong></td>
<td></td>
<td></td>
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<tr>
<td>AOT Step</td>
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<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sit</td>
<td>122 (20)</td>
<td>131 (24)</td>
<td>NS</td>
</tr>
<tr>
<td>Min</td>
<td>97 (22)***</td>
<td>93 (21)***</td>
<td>NS</td>
</tr>
<tr>
<td>Stand</td>
<td>117 (22)†††</td>
<td>118 (25)***†††</td>
<td>NS</td>
</tr>
<tr>
<td><strong>DBP, mmHg</strong></td>
<td></td>
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<tr>
<td>AOT Step</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sit</td>
<td>76 (15)</td>
<td>87 (19)</td>
<td>NS</td>
</tr>
<tr>
<td>Min</td>
<td>55 (14)***</td>
<td>58 (17)***</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>Stand</td>
<td>77 (17)†††</td>
<td>83 (21)†††</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means (SD). SBP, systolic blood pressure; DBP, diastolic blood pressure; sit, sitting; min, minimum value during the first 30 s after active standing up; stand, standing. Significance of main effects for AOT step and Drug Condition are shown. Significant difference in paired contrast at **P < 0.01 and ***P < 0.001 when compared with sit. Significant difference in paired contrast at †††P < 0.001 when compared with min.

RANO (P < 0.001). The main effects for Drug Condition and for AOT Step and pairwise comparisons are shown in Table 1 and Table 2.

**RF during AOT.** The RF of those 11 subjects whose data were included in the results was 0.24 Hz (SD 0.04) (range 0.20–0.32 Hz) before the vagal blockade, 0.27 Hz (SD 0.05) (range 0.21–0.35 Hz) after the vagal blockade, 0.27 Hz (SD 0.05) (range 0.21–0.35 Hz) before the sympathetic blockade, and 0.27 Hz (SD 0.05) (range 0.20–0.34 Hz) after the sympathetic blockade. Before the blockades, RF did not differ significantly between the test days. Sympathetic blockade did not have significant effects on RF, but vagal blockade increased RF slightly (P < 0.05).

**DISCUSSION**

In the present study, we used selective vagal and sympathetic blockades to evaluate the capability of HFP derived from the STFT method in tracking transient, vagally mediated changes during AOT. Our results showed that the STFT method was able to monitor the changes in vagal effects on the heart during AOT. In the drug-free condition, HFP showed a rapid decrease immediately after standing up, followed by a gradual increase to the level characteristic for the standing posture and remaining lower than in the sitting baseline posture. The present blocking experiment confirmed that the fast and slow HFP responses to standing up reflected changes in vagal effects on the heart.

We observed a rapid decrease in SBP and DBP 12 s after the onset of standing, followed by an immediate shortening in RRI length. The mean time delay between standing up and RRI_min varied from 13 to 20 s across the drug conditions. It has been previously documented that the rapid shortening in RRI length reflects arterial baroreflex compensation for the transient fall in SBP and DBP (4). We further observed an immediate decrease in HFP occurring simultaneously with the decrease in arterial blood pressure and the shortening in RRI length. Because we used the 25-s Hanning time window in calculations of power spectra, each instantaneous HFP value included information from both the preceding and following 12.5 s. The mean time delay between standing up and HFP_min varied from 10 to 17 s across the drug conditions. Thus HFP seemed to reach its local minimum approximately when information from the sitting posture was no longer included in the 25-s time window. The immediate drug-free decrease in HFP after the onset of the standing posture, indicating vagal withdrawal, is in line with results from previous studies using other time-frequency approaches, such as SDA (4, 25) and the smoothed Wigner-Ville transformation (22, 28, 29, 38).

We evaluated the vagal and sympathetic effects on HFP level at the three steps of AOT and the magnitude of the fast and slow HFP responses to standing up through the pharmacological blockades. The present findings were in general agreement with the vagal origin of HFP (2, 3, 12, 23, 31, 33) by showing major decreases in HFP at all steps of AOT. The present findings further showed that there was still some variation in the instantaneous HFP values after the vagal blockade, allowing us to detect HFP_min (see Fig. 1). However, after the vagal blockade, HFP_min did not differ from HFP_bl or HFP_stand. In contrast, the sympathetic blockade increased HFP_min and HFP_stand slightly, but significantly, but did not affect HFP behavior during the first 30 s after standing up. Because the sympathetic blockade increased HFP_min and HFP_stand, HFP in the standing posture was not completely independent of the magnitude of sympathetic activity. The increases were most likely due to a reflexive increase in vagal activity after the elimination of sympathetic activity, as suggested by the theory of reciprocal control of autonomic branches (7). The present results further showed that the magnitude of both HFP reactivity scores, i.e., the new fast and conventional slow HFP response to AOT, was abolished by the vagal blockade and unaffected by the sympathetic blockade (see Fig. 3). These findings suggest that HFP derived from the STFT method was able to detect and quantify a decrease in vagal activity during the unstable phase of AOT, where sudden changes occurred in arterial blood pressure and RRI length. The findings are in line with the presumed mechanism of the immediate response elicited by assuming a standing posture: Standing up results in a transient fall in arterial blood pressure, which is compensated by reflexive vagal inhibition and sympathetic activation (4, 18, 37). Because of the neural regulatory compensation, arterial blood pressure increases rapidly, causing rapid vagal activation and finally, stabilization of

Table 2. Blood pressure at the different steps of AOT before and after sympathetic blockade

<table>
<thead>
<tr>
<th></th>
<th>No Drug</th>
<th>Metoprolol</th>
<th>Drug Condition</th>
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<tr>
<td><strong>SBP, mmHg</strong></td>
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<td></td>
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<td>AOT Step</td>
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<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sit</td>
<td>111 (20)</td>
<td>113 (21)</td>
<td>NS</td>
</tr>
<tr>
<td>Min</td>
<td>85 (28)***</td>
<td>86 (32)***</td>
<td>NS</td>
</tr>
<tr>
<td>Stand</td>
<td>106 (22)†††</td>
<td>107 (25)***†††</td>
<td>NS</td>
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<td><strong>DBP, mmHg</strong></td>
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<tr>
<td>AOT Step</td>
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<tr>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td></td>
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</tr>
<tr>
<td>Sit</td>
<td>70 (11)</td>
<td>70 (13)</td>
<td>NS</td>
</tr>
<tr>
<td>Min</td>
<td>46 (14)***</td>
<td>47 (17)***</td>
<td>NS</td>
</tr>
<tr>
<td>Stand</td>
<td>69 (12)†††</td>
<td>69 (16)†††</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are means (SD). Significance of main effects for AOT step and Significant difference in paired contrast at *P < 0.05 and ***P < 0.001 when compared with sit. Significant difference in paired contrast at †††P < 0.001 when compared with min.
the sympathovagal balance characteristic for the standing posture (4, 18, 37).

Unlike the HFP responses to AOT, the RRI (or HR) responses did not provide independent information about either branch of the ANS in the drug-free condition because they reflect the intrinsic sinus node rate plus the sum of the influences of vagal and sympathetic nervous systems (26). However, the fast and slow RRI (or HR) responses to AOT provided information about separate vagal and sympathetic effects on the heart when pharmacological blockades were used. Our results showed that vagal and sympathetic blockades influenced the RRI reactivity scores in a similar manner as the HFP reactivity scores (see Fig. 3). In contrast, the HR reactivity scores were affected by both vagal and sympathetic blockades. Thus the present results demonstrated, as others have reported previously (12, 7, 9), that the use of RRI versus HR as a metric of cardiac chronotropy might lead to different interpretations of autonomic regulation underlying the cardiac response to AOT. The fast and slow cardiac responses to AOT seemed to be mediated by the vagal nervous system alone when interpreted from the RRI response, whereas both the vagal and sympathetic nervous system seemed to affect the cardiac responses to AOT when interpreted from the HR response. The different interpretations are caused by a nonlinear transformation of RRI to HR, as established in Fig. 4. Pragmatic and theoretical evidence supports the use of RRI as a cardiac chronotropic metric for both the tonic state and phasic response (9, 10, 12, 13, 19). A major advantage is that the transfer function between autonomic outflow and RRI is essentially linear, and thus a given millisecond change in RRI represents an equivalent change in autonomic outflow independent of the baseline state (7, 9). The present results regarding the HFP and RRI reactivity scores demonstrated that the HFP reactivity scores derived from the drug-free condition provided corresponding information about the autonomic regulation underlying the cardiac response to AOT as interpretation of the RRI response with pharmacological blockades.

**Limitations.** To obtain a high time resolution for HFP, we selected the 25-s time window for the STFT calculations and 0.20 Hz as the lower boundary of the high-frequency band, instead of the 0.15 Hz used in most studies. This decision induced some limitations. First, it is impossible to assess LFP by using such a short time window because the duration of the time window should be at least five times the slowest analyzed wavelength (4, 15, 25). Second, respiration may be distributed much wider than 0.20–0.40 Hz, and consequently, parts of the respiration-induced changes in RRI variability might be ignored in the calculations of HFP. However, in the present results, we included only the data of the subjects whose respiratory frequency ranged between 0.20 and 0.40 Hz. This meant that 7 subjects of the original 18 subjects were excluded from these results.

An additional limitation arises from spontaneous respiration. It is known that HFP does not accurately mirror vagal effects on the heart when there are changes in respiratory frequency and/or tidal volume (8, 20). In the present study, we did not control the respiration. Instead, as mentioned above, we selected afterward those subjects whose respiration was within the predetermined frequency range. Although the vagal blockade increased RP significantly, the mean and standard deviation of the RF after the vagal blockade were, however, identical to the means and standard deviations of the RF obtained before and after the sympathetic blockade. Thus it is unlikely that this slight, although significant, increase in RF would have had a major effect on HFP. Therefore, it seems obvious that the major decrease in HFP after the vagal blockade was caused by the blocking drug. Despite these limitations, the present results were able to demonstrate significantly the systematic behavior of HFP derived by the STFT method.

To avoid the side effects of pharmacological autonomic blockades, the drug and dosage applied in the present study were carefully chosen based on literature. The applied doses are generally considered to produce a complete blockade (6, 12, 24). However, physiological and psychological side effects of the drug may always influence autonomic modulation. Systematic biases may arise for example from interactions among sympathetic and vagal nervous systems, reflexive adjustments in the unblocked branch, nonselective actions of the blocking agent and incomplete blockade (6). Sympathetic blockade, for example, may alter arterial blood pressure and yield a baroreflex alteration of vagal activity (6). In the present study, neither the sympathetic nor vagal blockade affected SBP or DBP. The systematically, although nonsignificantly, higher SBP and DBP on the day when the vagal blockade was carried out might be explained by the “white coat” syndrome. Seven of the eleven subjects included in the results received atropine on the first test day and metoprolol on the second test day.

In summary, we evaluated the ability of HFP derived by the STFT method to detect the transient changes in vagal effects on the heart during AOT, a well-known external autonomic stimulus, by using selective vagal and sympathetic blockades. Our results from the drug-free condition, without controlled respiration, demonstrated that HFP rapidly decreased immediately after standing up and then gradually increased toward the level characteristic for the standing posture, remaining lower than in the sitting baseline posture. The results further demonstrated that the magnitude of the fast and slow HFP response to AOT was abolished by the vagal blockade and unaffected by the sympathetic blockade.
The present findings indicate that HFP derived by the STFT method provided a tool for monitoring the magnitude and time course of transient changes in vagal effects on the heart, without the need to interfere with normal control by using blocking drugs. Continuous research and development are needed to determine the most appropriate frequency range and time window for detecting a given phenomenon of interest.

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