Optimal frequency ranges for extracting information on cardiovascular autonomic control from the blood pressure and pulse interval spectrograms in mice

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Submitted 12 July 2006; accepted in final form 2 October 2006

Baudrie V, Laude D, Elghozi JL. Optimal frequency ranges for extracting information on cardiovascular autonomic control from the blood pressure and pulse interval spectrograms in mice. Am J Physiol Regul Integr Comp Physiol 292: R904–R912, 2007. First published October 19, 2006; doi:10.1152/ajpregu.00488.2006.—The analysis of blood pressure (BP) and heart rate (HR) variability by spectral methods has proven a useful tool in many animal species for the assessment of the vagal and sympathetic contributions to oscillations of BP and HR. Continuous BP measurements obtained in mice by telemetry were used to characterize the spectral bandwidths of autonomic relevance by using an approach with no a priori. The paradigm was based on the autonomic blockades obtained with conventional drugs (atropine, prazosin, atenolol). The spectral changes were estimated in all of the combinations of spectral bandwidths. The effect of hydralazine was also tested using the same systematic analysis, to detect the zones of sympathetic activation resulting reflexly from the vasodilatory action of the drug. Two zones of interest in the study of the autonomic control of BP and HR were observed. The first zone covered the 0.15–0.60 Hz range of the systolic BP spectrum and corresponds to the low-frequency zone (or Mayer waves). This zone reflects sympathetic control since the power spectral density of this zone was significantly reduced with α1-adrenoceptor blockade (prazosin), while it was significantly amplified as a result of a reflex sympathetic activation (hydralazine). The second zone covered the 2.5–5.0 Hz range of the pulse interval spectrum and corresponded to the high-frequency zone (respiratory sinus arrhythmia) under vagal control (blocked by atropine). These zones are recommended for testing the autonomic control of circulation in mice.

MATERIALS AND METHODS

Experiments were performed in eight adult male mice (C57BL/6; Charles River, L’Arbresle, France; 28 ± 2 g body wt) in accordance with the relevant guidelines of the French Ministry of Agriculture for scientific experimentation on animals and with European Communities Council Directive. Our personnel are authorized to conduct such investigations according to the Ministry’s Executive Order No. 75-215.

Surgery. Mice were anesthetized initially with 5% isoflurane in an oxygen stream and maintained on 2–3% isoflurane. Mice were kept on a heating pad throughout implantation of the BP telemeter (model TA11PA-C10; Data Sciences International, St. Paul, MN). The catheter was inserted into the left common carotid artery. This method has been previously described (5). The telemetric transmitter probe was positioned subcutaneously on the right flank. To reduce any infection and pain, the mice received one dose (20 mg/kg ip) of amoxicillin (Clamoxyl; SmithKlineBeecham Laboratories, Nanterre, France) and one dose (5 mg/kg ip) of ketoprofen (Profenid; Aventis, Paris, France). After the mice had recovered from the anesthesia in a warm (36°C) box, they were housed in individual cages placed on top of the telemetric receivers in a light-dark cycled recording room, for a 2-wk period to pressure transducers (14, 19, 21, 23, 34, 43). The responses to pharmacological blockade of the autonomic receptors support the view that spectral analysis of autonomic cardiovascular variability may be transferable to mice. However, the spectral bandwidths used to estimate the autonomic zones have been arbitrarily chosen and differ among the authors, leading to discrepant conclusions about the contribution of the autonomic components of BP and HR variability in this species. Consensual views, based on a series of decisive studies might lead to recommendations for bandwidths of importance for physiological studies, as happened with HR variability in humans (36a). An alternative was offered by Jaffe et al. (17), who analyzed all of the combinations of bandwidths of the HR spectrogram from one frequency resolution band to the entire spectrum to detect the zones responding to one stimulus (active orthostasis). This approach with no a priori was used in the present study. The paradigm was based on the autonomic pharmacological blockades obtained in mice with conventional drugs (atropine, prazosin, atenolol). The spectral changes were estimated in all of the combinations of spectral bandwidths. The bands affected by the blockades were assumed to represent the “autonomic” bands. The effect of hydralazine was also tested using the same systematic analysis, to detect the zones of sympathetic activation resulting reflexly from the vasodilatory action of the drug (11). All mice were equipped with radiotelemetric devices.

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Finally, simple statistics, i.e., mean and standard deviations of the distribution of the variables of the values of the 51.2-s segments used for the spectral analysis were computed.

**Statistical analysis.** Results are expressed as means ± SE. Paired t-tests were used to estimate the influence of treatments on systolic BP, PI, their variance, and their spectral frequency bands. Three levels of significance were used (P < 0.05, P < 0.01, and P < 0.001) to represent the effects of drugs on the different bands of the systolic BP and PI spectra. Since power values in adjacent frequency bands do not vary independently, a Bonferroni correction would have been too conservative. We therefore plot the P values below the conventional 0.05, 0.01, and 0.001 levels so that the degrees of significance are evident to the reader.

**RESULTS**

**Effects of treatments on systolic BP, PI, and their variances.**

Average systolic BP and PI levels are shown in Fig. 2. Saline injection did not significantly affect these variables. Prazosin significantly reduced systolic BP (−21 ± 4, from 113 ± 4 to 92 ± 3 mmHg, P < 0.01) and its variance (−11 ± 4, from 16 ± 4 to 5 ± 2 mmHg², P < 0.05). Atenolol induced a significant but moderate increase in PI (+11 ± 3, from 153 ± 5 to 164 ± 6 ms, P < 0.05). Atropine markedly reduced PI (−42 ± 9, from 146 ± 11 to 104 ± 4 ms, P < 0.01) with a marked reduction in PI variance (−47 ± 9, from 52 ± 10 to 5 ± 2 ms², P < 0.01) and an associated rise in systolic BP variance (+6 ± 3, from 9 ± 1 to 15 ± 3 mmHg², P < 0.05). Hydralazine induced a moderate systolic BP decrease (−9 ± 3, from 115 ± 6 to 106 ± 4 mmHg, P < 0.05).

**Spectral analysis of systolic BP and PI.** Two representations were used to illustrate the spectral changes with one quantitative representation indicating the amplitude of the changes according to the different bands of the spectra and a second qualitative representation indicating the significance of the changes according to the same bands. The first representation shows changes may be marked in the lowest frequency range where most of the power was concentrated. The qualitative picture shows zones of interest that are selectively affected by drug treatments. This representation was chosen to recognize relevant autonomic bands.

The effects of prazosin on systolic BP variability are illustrated by Fig. 3. This figure illustrates the quantitative (average) changes induced by α₁-adrenoceptor blockade with a zoom on the low-frequency (LF) bands. Changes (reduced power) are marked in the areas including frequency bands below 0.2 Hz with the highest changes for the bands including the very first frequency bands. Figure 3 also shows the qualitative value (significance) of these changes and a zoom on the LF range. The significant changes do not match the average changes, especially the lowest bands, which do not exhibit the highest significance. Conversely Figure 3 shows an area of significant changes in the high-frequency (HF) range, which are quantitatively small.

The zoom on the LF zone of interest shows the highest significance band (diamond), which corresponds to the 0.14–0.35 band (P < 0.001). The 0.15–0.60 Hz band is one band of high significance. Larger bands may include a higher fraction of the changes induced by the drug, but the significance of the changes for such bands is smaller. This is true for bands with a lower bound below 0.05 Hz, although lower bands include a large fraction of the changes.
Fig. 1. *Top*: example of a 30-min systolic blood pressure (BP) and pulse interval (PI) recording of a control period. Arrows delimit one period without erratic fluctuations, which is magnified below. The corresponding systolic BP and PI power spectra are shown at the bottom.

Fig. 2. Average levels of systolic BP (SBP) and PI of each session, before (white columns) and after (black columns) treatment obtained in 8 mice. Error bars correspond to means ± SE. *P < 0.05, **P < 0.01.
Hydralazine induced increases in the systolic BP power in bands including the lowest frequency ranges. The qualitative representation of its effects is shown in Fig. 4 including the zoom. Increases in power were observed in the LF range. The figure also shows that the zone of highest significance corresponds to the 0.10–0.49 band ($P < 0.001$). It is noticeable that the zoom on the LF changes resembles the zoom of the effects of prazosin. The 0.15- to 0.60-Hz band is within the highly significant area.

Atropine markedly affected the PI power spectrum as shown in Fig. 5. The PI power was reduced for all frequency combinations with the highest average changes for the lower bands. Although the average changes were quantitatively small in the HF range, the changes of highest significance covered HF bands with a lower bound of 2.5 Hz. The upper bound of the highly significant ($P < 0.001$) bands included the upper limit of the analyzed part of the spectra, i.e., 5 Hz. The zone of highest significance corresponded to the 2.56–4.69 band ($P < 0.001$). A 2.5 to 5.0 Hz bandwidth taking into account the upper bound of the highly significant band includes the usual rates of respiration of the mouse, which vary from 120 to 300 cycles/min. It also corresponds to a zone of highly significant spectral PI change. Atropine also significantly reduced the LF component of the PI spectrum. A significance below 0.01 covered the whole LF zone from 0.27 to 1.89 Hz. Atropine also affected the systolic BP spectrum with an increased power over the whole spectrum with the highest changes in the lower frequency bands and with significant changes in the HF range. The highest significance corresponded to the 1.45–3.55 band ($P < 0.001$), i.e., a band with a lower bound slightly below the lower bound of the PI spectrum described above and a higher bound slightly below the upper limit of the analyzed spectrum.

The effect of saline injection on the systolic BP spectrum is shown in Fig. 6. Changes were of minor amplitude, and the qualitative representation shows the changes reached significance in the HF (respiratory) range, although they never reached the 0.001 threshold, as was observed with other drugs.

The effects of atenolol on systolic BP and PI spectra and the effects of prazosin, hydralazine, and saline on PI spectra are not shown since the percentage of significant ($P < 0.05$) changes of the corresponding spectra never exceeded 3% of the total number of bandwidths combinations and most likely corresponded to sampling fluctuations.

Levels of systolic BP and PI powers were calculated after injections of saline or drugs in the frequency ranges identified in this study, i.e., 0.15–0.60 Hz range for LF and 2.5–5.0 Hz range for HF. Figure 7 illustrates these levels and include the significance of the changes vs. control levels. Prazosin reduced the LF systolic BP power, while hydralazine increased this component. Atropine reduced the LF and HF PI powers.
DISCUSSION

Autonomic drugs and hydralazine modified large zones of the BP and PI spectra of mice equipped with a telemetric device. A statistical approach restricted these zones to smaller bandwidths, which were more selective for detecting autonomic effects. We propose this approach to enable the definition of optimal bands of interest for studying the autonomic control of cardiovascular variables in conscious mice.

The BP and PI levels varied according to the selective action of drugs on the autonomic nervous system. It has to be pointed out that the resting HR was low (418 beats/min or 147 ms for PI) in these acclimatized mice recorded during a resting period (during the morning) by telemetry. These low levels of HR indicate the stress reaction to handling and injections was minimal, and this was illustrated with the dominant role of the vagus translated into marked HR changes with atropine, while the cardiac sympathetic control was minimal at rest, as shown with the minor effect of atenolol. The direct vasodilator hydralazine produced a diminution of systolic BP (−8.4%). The effect of the injection procedure was not detectable after 30 min, when the second recording was initiated, since the BP and PI recorded after saline injection were not significantly changed compared with the preinjection levels.

Fig. 4. Average systolic BP power spectral changes induced by hydralazine (left) and significance of these changes (right). The lower charts zoom on the changes in the LF range. The degree of systolic BP power spectral changes and the degree of significance are represented with the same colors as those shown in Fig. 3.

Fig. 5. Average PI power spectral changes induced with atropine on the left and significance of these changes on the right. The degree of PI power spectral changes are represented by the scale of colors, and the degree of significance with the 3 colors is shown.
The overall variability of BP reflected by the variance of systolic BP was diminished by prazosin (−67%) and increased by atropine (+65%). The contribution of the vascular sympathetic to the short-term BP variability (including Mayer waves) underlies the effect of prazosin. The opposite effects of atropine on BP and PI variance recall a previous study in rats by Ferrari et al. (9). These authors demonstrated an anti-oscillatory role of the vagus on BP (9). The blockade of the vascular fluctuations of the heart by atropine resulted in a marked reduction in the PI variance (−91%) in mice. A loss of the vagal cardiac fluctuations usually buffering BP changes may then indirectly determine an increase in BP variance.

The spectral analysis documented the effects of drugs on systolic BP and PI variability in the frequency domain. Two zones of significant variations were observed in mice with one on the systolic BP spectra covering roughly the 0.15–0.60 Hz range that could be called the LF range and one detected on the PI spectrum covering the 2.5–5.0 Hz range that could be called the HF range. It has to be stressed that the amplitude of the changes were not necessarily high to reach significance and this was obvious for the HF component of the PI spectrum. In contrast, the average changes were marked in the lowest frequency ranges corresponding to the very LF (VLF) component (see below), where these changes did not reach significance. Prazosin markedly reduced the LF component in systolic BP spectrum, whereas hydralazine increased it. Similar changes were previously reported with prazosin (20) or phentolamine (6) and hydralazine (11) in rats and more recently with prazosin in mice (19, 23). These effects may well represent the consequence of the treatments on the so-called Mayer waves (22). Prazosin attenuated these systolic BP oscillations by blocking the vascular transducer (vascular α1-adrenoceptors) of the oscillations of the sympathetic nervous system. The amplification of these oscillations with hydralazine may parallel the reflex sympathetic activation following vasodilatation. These reflex changes were maintained although carotid sinus baroreceptors were functionally impaired on one side, inherent in the arterial catheterization procedure.

The effects of atropine on the PI spectrum may reflect the vagal blockade of heart rhythm fluctuations associated with respiration, which is called the respiratory sinus arrhythmia. This effect was reported in rats (6, 20) and many other species including man (24, 27, 38). The average respiratory rhythm normally fluctuates between 1.5 and 5 Hz in conscious mice, and this determined the width of this component. Average respiratory rhythms between 2.5 and 3.5 Hz have been reported

Fig. 6. Average systolic BP power spectral changes induced with physiological saline (left) and significance of these changes (right). See scale of colors in Fig. 3.

Fig. 7. Levels of SBP and PI spectral powers after injections of saline or drugs in the LF (left) and HF (right) ranges as defined in the text, i.e., 0.15–0.60 Hz for LF and 2.5–5.0 Hz for HF. *P < 0.05, ***P < 0.001 vs. control levels.
(14, 34, 35, 37). Uechi et al. (37) directly measured respiratory rate of conscious mice using piezoelectric ultrasonic crystals implanted on opposite surfaces of the chest. These authors also showed the power spectra of respiration was coupled closely with the HF component of the ECG power spectra (37).

Effects of atropine were also observed in the LF range of the PI spectrum, although less marked, as previously reported in rats (6, 20). This effect reflects the predominant role of the vagus in all of the frequency ranges, and this confirms previous observations in rats and other species, including man, that HR fluctuations in the LF range also depend on the vagus (3, 6, 20, 38). Atenolol did not affect the PI spectrum, suggesting a minor contribution of the cardiac sympathetic nerves to HR variability in mice under our resting conditions (see below).

The two autonomic bands detected in this study may represent the optimal bands for studying the autonomic effects of various (genetic) manipulations or treatments. Previous studies have explored this autonomic control of circulation using different frequency bands. In our seminal paper on the autonomic zones in rats (20), the areas of the spectra were divided into three zones, corresponding to what is now commonly called the VLF component, the LF component corresponding to Mayer waves, and the HF (respiratory) component. Some authors call the LF component the midfrequency component (14, 19, 34), and some divide the LF component into two bands, analyzing up to five different zones (19). The present data favor the use of only two bands, as far as the autonomic nervous system is concerned, but the present study does not explore the nonautonomic components of cardiovascular variability. An example is the study performed by Stauss et al. (34), who showed that endothelial nitric oxide modulates BP variability in a frequency range impinging on the LF and VLF domain. The spectral zones analyzed by a selection of spectral studies in mice are listed in Table 1. This table illustrates the wide dispersion of the zones explored by previous authors.

The main limitation of the study is related to the mouse species that is now largely used in genetics. The description of its autonomic cardiovascular control requires periods of cardiovascular stability, which are scarce and of short duration in this species. Janssens et al. (18) showed that rapid and frequent behaviorally induced changes in venous return influence cardiac output and BP in mice. In the present study, the conditions for continuous BP recordings with minimal arousal were present; telemetry was used with a long delay for recovery, and recordings were made in a dedicated room after an acclimatization period, during the morning when the mice would normally be asleep. HR was low (average value: 418 beats/min) compared with most of the average HR values reported by others, and close to the values reported by Pelat et al. (32) (440 beats/min) and Chen et al. (7) (451 beats/min) based on BP telemetric recordings with a long delay for recovery. Due to the design of this study, arousal was minimized, and this could also explain why the sympathetic control of HR was minimal, reflected by a limited effect of atenolol on HR, compared with the dominant vagal control of HR, reflected by the dramatic effects of atropine.

The relative roles of the parasympathetic vs. sympathetic nervous systems in control of HR remain controversial in mice. The authors reporting the highest levels of resting HR (from 647 up to 724 beats/min) (10, 19, 23, 39) did not observe significant effects of atropine on HR, while those reporting the lowest levels, i.e., Pelat et al. (440 beats/min) (32), Fazan et al. (8) (531 beats/min) (5) and Gross et al. (13) (560 beats/min) reported profound effects of atropine on the HR and power spectra similar to our observations, i.e., a HR rise associated with decreases of the two main (LF and HF) HR power spectral components. In contrast, the effects of beta blockers on HR were quantitatively important in studies reporting elevated HR levels. In other words, the lower the HR the higher the vagal control and vice versa for the cardiac sympathetic control. The recording design largely determines the HR levels. Using the telemetric procedure, with a 1-wk recovery between the operation and the recording, we found that the average HR levels were 440–550 beats/min during the morning. The lower HR may reflect more physiological HR levels with minimized consequences of the operative procedure and an acclimatization to the recording conditions (isolation, environment of the recording room). Mice also exhibit the lowest HR during the

Table 1. List of the authors analyzing spectral zones of potential cardiovascular relevance in the conscious mice

<table>
<thead>
<tr>
<th>Author</th>
<th>Reference No.</th>
<th>Procedure</th>
<th>Variable</th>
<th>Zones Selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stauss et al.</td>
<td>34</td>
<td>BP catheter</td>
<td>MBP</td>
<td>LF 0.05–0.4 Hz, MF 0.4–0.8 Hz, HF ±0.5 Hz around the respiration peak</td>
</tr>
<tr>
<td>Janssens et al.</td>
<td>19</td>
<td>BP catheter</td>
<td>MBP, PI</td>
<td>VLF&lt;0.08 Hz, LF1 0.08–0.4 Hz, LF2 0.4–1 Hz, MF 1–3 Hz, HF 3–10 Hz</td>
</tr>
<tr>
<td>Just et al.</td>
<td>23</td>
<td>BP catheter</td>
<td>MBP, PI</td>
<td>VLF&lt;0.15 Hz, LF&lt;0.15–1.5 Hz, HF 1.5–5 Hz</td>
</tr>
<tr>
<td>Head et al.</td>
<td>14</td>
<td>BP catheter</td>
<td>MBP, PR</td>
<td>LF 0.08–0.3 Hz, MF 0.3–0.5 Hz, HF 0.5–1 Hz</td>
</tr>
<tr>
<td>Joaquim et al.</td>
<td>21</td>
<td>BP catheter</td>
<td>MBP, PI</td>
<td>LF 0.1–1 Hz, HF 1–5 Hz</td>
</tr>
<tr>
<td>Xue et al.</td>
<td>43</td>
<td>BP catheter</td>
<td>PI</td>
<td>LF 0.1–1.75 Hz, HF 1.75–5 Hz</td>
</tr>
<tr>
<td>Gross et al.</td>
<td>12</td>
<td>BP telemetry</td>
<td>SBP, PI</td>
<td>VLF 0.015–0.25 Hz, LF 0.25–0.6 Hz, HF 1–3 Hz</td>
</tr>
<tr>
<td>Pelat et al.</td>
<td>32</td>
<td>BP telemetry</td>
<td>SBP, PR</td>
<td>VLF 0.05–0.4 Hz, LF 0.4–1.5 Hz, HF 1.5–5 Hz</td>
</tr>
<tr>
<td>Williams et al.</td>
<td>40</td>
<td>BP telemetry</td>
<td>SBP</td>
<td>LF 0.4–1.5 Hz, HF 1.5–4 Hz</td>
</tr>
<tr>
<td>Witte et al.</td>
<td>42</td>
<td>BP telemetry</td>
<td>SBP, PI</td>
<td>LF 0.08–1.5 Hz, HF 1.5–5 Hz</td>
</tr>
<tr>
<td>Ishii et al.</td>
<td>16</td>
<td>ECG telemetry</td>
<td>RR</td>
<td>LF 0.1–1 Hz, HF 1–5 Hz</td>
</tr>
<tr>
<td>Mansier et al.</td>
<td>26</td>
<td>ECG telemetry</td>
<td>RR</td>
<td>LF 0.4–1.1 Hz, HF 1.4–4 Hz</td>
</tr>
<tr>
<td>Uechi et al.</td>
<td>37</td>
<td>ECG telemetry</td>
<td>RR</td>
<td>LF 0.1–1.75, HF 1.75–5 Hz</td>
</tr>
<tr>
<td>Wickman et al.</td>
<td>39</td>
<td>ECG telemetry</td>
<td>RR</td>
<td>VLF&lt;0.4 Hz, LF 0.4–1.5 Hz, HF 1.5–5 Hz</td>
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<tr>
<td>Gehmann et al.</td>
<td>10</td>
<td>ECG telemetry</td>
<td>RR</td>
<td>LF 0.4–1.5 Hz, HF 1.5–4 Hz</td>
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<tr>
<td>Holschneider et al.</td>
<td>15</td>
<td>ECG telemetry</td>
<td>RR</td>
<td>VLF 0.03–0.08 Hz, LF 0.08–1 Hz, HF 1–4 Hz</td>
</tr>
<tr>
<td>Tankersley et al.</td>
<td>36</td>
<td>ECG telemetry</td>
<td>RR</td>
<td>LF 0.2–1.5 Hz, 1.5–5</td>
</tr>
</tbody>
</table>

BP, blood pressure; MBP, mean BP; PI, pulse interval; PR, pulse rate; RR, RR interval; HR, heart rate; LF, low frequency; MF, midfrequency; HF, high frequency; VLF, very LF.
morning in relation to a circadian HR rhythm (12, 19, 32, 41, 42). The choice of the morning for recording cardiovascular variables may favor vagal dominance. Under our conditions, the effects of atenolol were limited to a slight reduction in HR. Effects of atenolol on the PI spectrum were negligible. This is in line with many papers reporting that β₁-adrenergic inhibition had only small effects on the PI spectrum, despite a depression of mean HR (8, 13, 19, 23).

The specific analysis of the sympathetic component of the HR spectrum would require another study on the effects of the beta blocker at night or during exposure to a stressful condition, such as a novel environment which increases HR (15).

Whatever care is given to the recording protocol, periods without erratic fluctuations are scarce and of short duration in mice, limiting the application of the frequency-domain analysis based on the Fourier transform. Other techniques, such as time/frequency domain procedures, might be adapted to these signals in the future, as long as these techniques provide a good resolution at the “autonomic” frequency ranges, independently if signals remain stationary.

The main features of this study can be summarized as follows. Continuous BP measurements obtained in mice by telemetry showed two zones of interest in the study of the autonomic control of BP and HR. The first zone covers the 0.15–0.60 Hz range of the systolic BP spectrum and corresponds to the LF zone (or Mayer waves). This zone reflects sympathetic control since the power spectral density of this zone is significantly reduced with α₁-adrenoceptor blockade (prazosin), whereas it is significantly amplified as a result of a reflex sympathetic activation (hydralazine). The second zone covers the 2.5–5.0 Hz range of the PI spectrum and corresponds to the HF zone (respiratory sinus arrhythmia) under a vagal control as it is markedly affected by atropine. These zones are recommended for testing the autonomic control of circulation in mice.

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31. Pelat M, Dessy C, Massion P, Desager JP, Feron O, Balligand JL. Rosuvastatin decreases caveolin-1 and improves nitric oxide-dependent


