Arterial pressure fluctuates rhythmically in healthy supine resting humans, who, from all outward appearances, are in a ‘steady-state’. Others have asked, if baroreflex mechanisms are functioning normally, how can arterial pressure be so variable? We reanalysed data from nine healthy young adult men and women and tested the hypotheses that during brief periods of observation, human baroreflex sensitivity fluctuates widely and rhythmically. We estimated vagal baroreflex sensitivity with systolic pressure and R–R interval cross-spectra measured over 15 s segments, moved by 2 s steps through 20-min periods of frequency- and tidal volume-controlled breathing. We studied each subject at the same time on three separate days, with fixed protocols that included two physiological states, supine and passive 40 deg upright tilt, before and after β-adrenergic, cholinergic, and angiotensin converting enzyme blockade. Minimum, mean and maximum (± S.D.) supine control baroreflex sensitivities averaged 5 ± 3, 18 ± 6, and 55 ± 22 ms mmHg−1.

In most subjects, moderate ongoing fluctuations of baroreflex sensitivity were punctuated by brief major peaks, yielding frequency distributions that were skewed positively. Fast Fourier transforms indicated that baroreflex sensitivity fluctuations (expressed as percentages of total power) concentrated more in very low, 0.003–0.04 Hz, than ultra low, 0.0–0.003 Hz, frequencies (77 ± 7 versus 11 ± 8%, P ≤ 0.001, rank sum test). Autoregressive centre frequencies averaged 0.012 ± 0.003 Hz. The periodicity of very low frequency baroreflex sensitivity fluctuations was not influenced significantly by upright tilt, or by variations of autonomic drive or angiotensin activity. Our analysis indicates that during ostensibly ‘steady-state’ conditions, human vagal baroreflex sensitivity fluctuates in a major way, at very low frequencies.

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DOI: 10.1113/jphysiol.2005.091090
Methods

Subjects

The experiment we reanalysed (Taylor et al. 1998) explored the role of the renin–angiotensin–aldosterone system in modulating human heart rate variability. Six men and three women, ages 23–28 years, gave written informed consent to participate in the study, which was approved by the human research committees of the Hunter Holmes McGuire Department of Veterans Affairs Medical Center and Virginia Commonwealth University and conformed to the Declaration of Helsinki. All subjects were healthy and none were taking medications. Subjects abstained from alcohol and caffeine ingestion and strenuous physical exercise for 24 h prior to the experiments.

Protocol

Studies were conducted at the same time on three separate days, with intravenous injections given in fixed orders. Day 1: saline (control); the hydrophilic $\beta$-adrenergic blocking drug, atenolol, 0.2 mg kg$^{-1}$; and the muscarinic cholinergic blocking drug, atropine sulphate, 0.04 mg kg$^{-1}$. Day 2: saline, atropine, and atenolol. Day 3: saline, and the angiotensin converting enzyme blocking drug, enalaprilat, 0.02 mg kg$^{-1}$. We made measurements with subjects in the supine and 40 deg passive head-up tilt positions, before and after each injection. Trained subjects breathed at 0.25 Hz (15 breaths min$^{-1}$) at a comfortable tidal volume, which each established during quiet breathing at the beginning of the first experimental session.

Measurements

We recorded the electrocardiogram, finger photoplethysmographic arterial pressure (Finapres Model 2300, Ohmeda, Englewood, CO, USA), tidal volume (Fleisch pneumotachograph), and end-tidal carbon dioxide concentration (infrared analyser connected to a port in a face mask). We recorded data on FM tape and subsequently digitized them at 500 Hz with Windaq hardware and software (Dataq Instruments, Akron, OH, USA), for analysis with WinCPRS software (Absolute Aliens Oy, Turku, Finland).

Analyses

One author overread the WinCPRS detection of electrocardiographic R waves and systolic pressures and corrected errors. We estimated vagal baroreflex sensitivity three ways. First, we integrated power spectra of systolic pressure and R–R intervals within the frequency range, 0.04–0.15 Hz, and considered baroreflex sensitivity to be the square root of the ratio between R–R interval and systolic pressure integrated spectra (the ‘$\alpha$-coefficient’; Pagani et al. 1988). Second, we performed the same analysis, but only when the coherence was $\geq 0.50$ and the phase was negative (that is, systolic pressure changes probably led R–R interval changes) within this frequency range (Badra et al. 2001). To obtain moving baroreflex sensitivity estimates, we iteratively made measurements from a brief duration window (see Results), moved by steps through each 20 min data collection period. Time series generated by these calculations were evaluated with fast Fourier transforms to quantify power in the ultra low and very low frequency regions, and with autoregression (with a fixed model order of 20) to determine the centre frequencies of oscillations.

Third, we estimated baroreflex sensitivity with the ‘sequence method’ (Bertinieri et al. 1985; Fritsch et al. 1986), which is based on the assumption (supported by measurements made before and after sinoaortic baroreceptor denervation; di Rienzo et al. 1991) that parallel upgoing and downgoing pairs of systolic pressures and R–R intervals are expressions of spontaneous baroreflex physiology. We used parameters derived from an earlier analysis (Rothlisberger et al. 2003), and required that valid sequences comprise three or more pairs of systolic pressures increasing or decreasing by at least 1 mmHg, and R–R intervals lengthening or shortening by at least 5 ms per beat. If a linear regression analysis of such three or more systolic pressure–R–R interval pairs yielded a correlation coefficient $\geq 0.80$, we accepted its slope as an index of baroreflex sensitivity.

We express results as means ± s.d. We compared measurements made in two circumstances, such as during supine rest and upright tilt, with Student’s t test. (When data were not distributed normally, we used the Mann–Whitney rank sum test.) We performed post hoc analyses of serial measurements with the Holm-Sidak test. We sought correlations among measurements with linear regression. All analyses were performed with SigmaStat 3.10 (Systat Software, Point Richmond, CA, USA). We considered $P \leq 0.05$ to be significant.

Results

Subjects controlled their breathing well, but not perfectly. The average breathing interval was 4 s (0.25 Hz, or 15 breaths min$^{-1}$), with an average standard deviation of only 0.06 s. However, most subjects had sighs, and unknown to the investigators, began controlled breathing at a slightly hypocapnic average level, 4.9 ± 0.1, and reduced their carbon dioxide levels further, but insignificantly ($P = 0.087$), to 4.5 ± 0.2%, by the end of 20 min controlled breathing periods. Average end-tidal carbon dioxide changes were not significantly different across experimental sessions ($P = 0.53$).
Preliminary analyses

Figure 1 shows moving baroreflex sensitivities calculated over different window widths (left panels, grey areas), average baroreflex sensitivities (left panels, horizontal lines), and their autoregressive spectra (right) for one subject. These analyses make three points. First, the window duration exerts no major influence on the average level of baroreflex sensitivity. Second, baroreflex sensitivity fluctuates importantly and quasi-periodically over brief periods of observation. Third, as expected, these baroreflex peaks dampen considerably as the width of the window during which a baroreflex calculation is made increases; however, the basic rhythmicity appears to be independent of window width, at least over the window widths we examined. We settled upon a 15 s window, moved by 2 s steps for all subsequent analyses, in part because this window duration enables us to report reliably on very low frequency oscillations, up to 0.033 Hz $1/(2 \times 15 \text{s})$, or one oscillation every 30 s.

Figure 2 depicts measurements made from one supine subject during the second recording session. This subject, for unknown reasons, experienced increases of systolic pressure, R–R interval, and baroreflex sensitivity during the 20 min recording. The bottom left panel shows cross-spectral (grey area), upgoing baroreflex sequences (circles, plotted at the times they began), and average baroreflex sensitivities (upsloping grey and black lines). The calculated centre frequency (right panel) was 0.011 Hz. In this subject, fluctuations of both cross-spectral and sequence baroreflex sensitivities were large. Moreover, over this 20-min period of observation, cross-spectral baroreflex sensitivity increased nearly threefold.

Main results

Table 1 lists supine control measurements from all subjects, averaged over the three recording sessions (there were no significant differences among measurements made on the three study days). Mean upgoing sequence and cross-spectral baroreflex sensitivities were identical, $18 \pm 4$ and $18 \pm 5 \text{ms mmHg}^{-1}$ ($P = 0.77$); however, both were significantly greater than downgoing sequences ($P = 0.02$ and 0.01). The range of cross-spectral baroreflex sensitivities averaged $50 \pm 19 \text{ms mmHg}^{-1}$, and exceeded the mean value in all subjects. The ratio of maximum to minimum baroreflex sensitivities averaged 14, and varied from 4 to 35 among subjects and study days. There were loose but significant correlations between baroreflex sensitivity, and baroreflex range ($r = 0.67, P = 0.05$) and very low frequency R–R interval spectral power ($r = 0.51, P < 0.001$). Mean baroreflex sensitivity calculated with the $\alpha$-coefficient was insignificantly lower than that calculated when coherence between R–R intervals and systolic pressures was > 0.50 and the phase was negative ($18 \pm 6$ versus $21 \pm 8 \text{ms mmHg}^{-1}$, $P = 0.1$).

Baroreflex sensitivity variability

As Fig. 2 indicates, there may be major, and in the case of this one subject during this one session, systematic changes of baroreflex sensitivity during a 20 min recording period. Average frequency distributions and cumulative probabilities for baroreflex sensitivity measured from this same subject during each supine recording session are shown in Fig. 3. All frequency distributions (upper panel) were positively skewed. Moreover, the flatness of these relations (kurtosis) varied greatly, from positive on Day 1

![Image of baroreflex oscillations](image_url)
Table 1. Average results from all volunteers in the supine position before drugs

<table>
<thead>
<tr>
<th>Subject</th>
<th>R–R interval (ms)</th>
<th>Arterial pressure (mmHg)</th>
<th>Baroreflex sensitivity (ms/mmHg)</th>
<th>Centre frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Sequence</td>
<td>Cross-spectral</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Up range</td>
<td>Down range</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Up</td>
<td>Down</td>
</tr>
<tr>
<td>A</td>
<td>901</td>
<td>104/51</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>B</td>
<td>893</td>
<td>148/70</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>C</td>
<td>1016</td>
<td>154/88</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>D</td>
<td>815</td>
<td>114/57</td>
<td>17</td>
<td>11</td>
</tr>
<tr>
<td>E</td>
<td>976</td>
<td>132/69</td>
<td>20</td>
<td>9</td>
</tr>
<tr>
<td>F</td>
<td>1026</td>
<td>134/66</td>
<td>14</td>
<td>8</td>
</tr>
<tr>
<td>G</td>
<td>822</td>
<td>107/68</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>H</td>
<td>1009</td>
<td>119/67</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>I</td>
<td>740</td>
<td>119/63</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>Mean</td>
<td>911</td>
<td>126/67</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>S.D.</td>
<td>103</td>
<td>18/10</td>
<td>4</td>
<td>6</td>
</tr>
</tbody>
</table>

Average of each day’s measurements for all subjects. Up and cross-spectral baroreflex sensitivities were similar ($P = 0.1$), but both were greater than down baroreflex sensitivities ($P = 0.02$ and 0.01). Ranges of up and cross-spectral baroreflex ranges also were similar ($P = 0.39$).

to negative on Days 2 and 3. In this subject, cross-spectral baroreflex sensitivities averaged $23 \pm 13$, $23 \pm 11$, and $34 \pm 11$ ms mmHg$^{-1}$ on Days 1, 2 and 3. Although average baroreflex sensitivities on Days 1 and 2 were identical, their frequency distributions and cumulative probabilities (Fig. 3, lower panel) were strikingly different.

Mean cross-spectral baroreflex sensitivities for the entire group were similar across experimental sessions, and averaged $18 \pm 5$, $17 \pm 5$ and $19 \pm 7$ ms ($P = 0.32$). Notwithstanding similar average baroreflex sensitivities, the degrees of skewness and kurtosis were highly variable. Frequency distributions for all subjects are shown in Fig. 4.

Figure 2. Measurements made from one supine subject

In the bottom left panel, cross-spectral baroreflex sensitivity is shown in grey, and up baroreflex sequences are shown as black circles. Average levels of baroreflex sensitivity are shown by the grey (cross-spectral) and black (sequence) lines. It is unclear why this resting, supine subject experienced increases of systolic pressures, R–R intervals, and baroreflex sensitivity during this 20 min recording.
All were positively skewed, such that even in volunteers with low average baroreflex sensitivities (such as Subject 1), baroreflex sensitivity could rise occasionally to very high levels.

Figure 5, left, shows moving baroreflex sensitivity estimates and average levels – horizontal lines – from one subject in the supine and tilted positions before drugs, and in the supine position after atenolol, enalaprilat, and atropine sulphate. Mean levels of baroreflex sensitivity varied substantially, according to the treatments. Although the centre frequencies of autoregressive peaks (right panels) varied somewhat in this subject, all were distinct, and all fell within the very low frequency range.

Average baroreflex sensitivities, centre frequencies, and very low frequency spectral power for all subjects for these interventions are given in Table 2. (Maximum frequencies were 0.0 during four (two subjects on each of two days) of the 27 experimental sessions.) There were no significant differences among very low frequency R–R interval spectral power divided by total R–R interval spectral power (VLF/Total).

Discussion

We iteratively estimated vagal baroreflex sensitivity in nine healthy young adults, during 20-min periods of frequency- and tidal volume-controlled breathing. Our analyses provide several new insights into human baroreflex physiology. First, baroreflex sensitivity is highly variable from minute to minute in subjects thought to be in a ‘steady-state’. Baroreflex variability is expressed as major ongoing fluctuations, but also by major variability of day-to-day baroreflex distributions. Second, baroreflex variability is organized, with oscillations occurring at very low frequencies of about 0.01 Hz, or once every 90 s. Third, despite changes of baroreflex sensitivity provoked by changes of posture or β-adrenergic, angiotensin converting enzyme, or cholinergic blockade, very low frequency baroreflex rhythmicity is preserved. Finally, baroreflex variability may have practical consequences in the regulation of arterial pressure (Appendix). These results make the case that the dimension, time, should be added to characterizations of human baroreflex function. The results also make the case that baroreflex rhythms have clinical relevance—they link prognostically important very low frequency R–R interval variability with prognostically important baroreflex physiology.

Ours is not the first study to document variability of human baroreflex function. Vagal baroreflex sensitivity may be higher during sleep than wakefulness (Smyth et al. 1969; Parati et al. 1988), may vary from one day to another (Eckberg, 1977), from time to time during the same experimental session (this study and Golenhofen & Hildebrandt, 1958; Yamamoto et al. 1989; Badra et al. 2001), and from rest to other physiological states, including upright tilt and physical exercise (Pickering et al. 1971), and mental arithmetic (Steptoe & Sawada, 1989).

The results we obtained from resting awake subjects place baroreflex variability in a new context: the variability we report appears to be a fundamental property of baroreflex physiology. Major baroreflex fluctuations occurred in all of our nine subjects, and were independent of the method used to estimate baroreflex sensitivity. A corollary of this observation is that the wide scatter of up and down baroreflex sequences (Fig. 2, Table 1) reflects true variability of baroreflex sensitivity and not noise. Fluctuations of baroreflex sensitivity are large: in supine subjects, the ratio of maximum to minimum baroreflex sensitivity ranged from 4 to 35! Baroreflex variability is expressed also in the distribution of baroreflex responses; measurements obtained on different days with identical average baroreflex sensitivities may have vastly different distributions. (All of our subjects had mean baroreflex sensitivities that were within 1 ms mmHg⁻¹ on at least two of the three study days.) On some days, baroreflex sensitivities vary within relatively narrow limits, and on
Table 2. Average baroreflex sensitivities and centre frequencies for all interventions

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Baroreflex sensitivity (ms/mmHg)</th>
<th>Centre frequency (Hz)</th>
<th>VLF Spectral power (ms/mmHg)²/Hz</th>
<th>VLF/Total spectral power (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supine</td>
<td>18 ± 5a,b</td>
<td>0.012 ± 0.003</td>
<td>36 ± 34</td>
<td>76 ± 7</td>
</tr>
<tr>
<td>Tilt</td>
<td>10 ± 4a</td>
<td>0.018 ± 0.008</td>
<td>10 ± 12</td>
<td>80 ± 5</td>
</tr>
<tr>
<td>Atenolol</td>
<td>25 ± 13a,b</td>
<td>0.016 ± 0.003</td>
<td>92 ± 131c</td>
<td>76 ± 4</td>
</tr>
<tr>
<td>Enalaprilat</td>
<td>19 ± 9a,b</td>
<td>0.010 ± 0.003</td>
<td>24 ± 15</td>
<td>75 ± 8</td>
</tr>
<tr>
<td>Atropine sulphate</td>
<td>1.6 ± 0.6</td>
<td>0.010 ± 0.003</td>
<td>0.1 ± 0.01</td>
<td>68 ± 26</td>
</tr>
</tbody>
</table>

Centre frequencies were derived from autoregression analyses, with a fixed model order of 20. VLF/Total = integrated R–R interval fast Fourier transform spectral power between 0.003 and 0.04 Hz divided by total spectral power between 0 and 0.05 Hz. Statistically significant (P < 0.05) differences: a greater than atropine; b greater than tilt; c greater than tilt and atropine. There were no significant differences among centre frequencies (0.71) or VLF/Total spectral powers (P = 0.32).

During each session, baroreflex sensitivity varied widely. All subjects experienced episodic, very high baroreflex sensitivity.
In this subject, although upright tilt and blocking drugs altered the magnitudes of baroreflex sensitivity fluctuations, they did not appear to alter baroreflex rhythmicity.

In this figure, systolic pressure is superimposed on moving cross-spectral baroreflex sensitivity (shown in grey, left). Each right panel represents signal-averaged systolic pressures, triggered on mean baroreflex sensitivity, and 2, 4, 6 and 8 ms mmHg\(^{-1}\) below the mean level. Gradations of grey are from the highest baroreflex sensitivity (the lightest shade of grey) to 8 ms mmHg\(^{-1}\) below the mean level of baroreflex sensitivity (the darkest line). These data suggest that in these subjects, reductions of baroreflex sensitivity caused elevations of systolic pressure.
No new fourier transform spectral power also aggregated in the very low frequency range. Our subjects’ breathing control was not perfect; however, although the small reductions of carbon dioxide that we documented probably altered subjects’ responses (Henry et al. 1998), they are unlikely to have provoked the major changes of R–R interval fluctuations that would have attended small changes of breathing frequency, absent breathing control (Brown et al. 1993). Moreover, changes of end-tidal carbon dioxide levels occurring during 20 min recordings were similar for all interventions. Voluntary control of breathing is unlikely to have influenced our results (Patwardhan et al. 1995).

Clinical implications

Cardiovascular diseases are associated with diminished vagal baroreflex sensitivity (Eckberg et al. 1971) and diminished vagal–cardiac and augmented sympathetic–muscle neural outflows (Leimbach et al. 1986; Porter et al. 1990), in inverse proportion to the severity of disease. The prognosis in animals and humans with heart disease is poor when vagal baroreflex sensitivity (Billman et al. 1982; LaRovere et al. 1998) or vagally mediated heart rate variability (Bigger et al. 1992; Huikuri et al. 1995) is low.

Of particular relevance are the observations of Bigger et al. (1992) and Huikuri et al. (1995), that diminished very low frequency heart rate variability portends an especially bad prognosis. Our study ties together very low frequency heart rate variability and arterial baroreflex function. It may be therefore that the distinction drawn in some studies between heart rate variability and baroreflex sensitivity is artificial—heart rate variability and baroreflex mechanisms may be closely intertwined.

Appendix

Two subjects had stable pressure levels which, for unknown reasons, were interrupted episodically by 10–20 mmHg systolic pressure elevations. These subjects were different from other subjects, who tended to have systolic pressures that simply oscillated above and below their mean levels. The left panels of Fig. 6 indicate that elevations of systolic pressures tended to occur at times when vagal baroreflex sensitivity was low (troughs in the grey areas). The right panels of Fig. 6 depict systolic pressures during these 20-min periods, signal-averaged on cross-spectral baroreflex sensitivity. The lightest relations represent systolic pressure signal-averaged on mean baroreflex sensitivity, and increasingly dark relations represent systolic pressure changes provoked by successively lowered (by 2 ms mmHg$^{-1}$) baroreflex thresholds. In both subjects, systolic pressure elevations tended to be greater as the baroreflex threshold crossing was lowered.

Many studies document inverse relations between vagal baroreflex sensitivity and arterial pressure fluctuations. In patients with longstanding hypertension, blood pressure variability is greatest in those with the lowest baroreflex sensitivity (Mancia et al. 1983, 1985), and in 24 h recordings from healthy and hypertensive subjects, reductions of baroreflex sensitivity during activities of daily living are associated with increases of blood pressure and blood pressure variability (Watson et al. 1980; Tochikubo et al. 1987; Floras et al. 1988; Parati et al. 1988). The preliminary analyses discussed above may extend these earlier observations by indicating that at least in some subjects, reductions of vagal baroreflex sensitivity lasting only tens of seconds provoke increases of arterial pressures. This raises the possibility that short-term baroreflex variability is not an artefact, but rather, an oscillation that carries true physiological significance.

References


**Acknowledgements**

We thank J. Andrew Taylor, Michael A. Cohen, and Istvan Bonyhay for their thoughtful comments regarding this project. This research was supported by longstanding grants and contracts from the National Institutes of Health, the Department of Veterans Affairs, and the National Aeronautics and Space Administration.