Mechanisms Underlying Very-Low-Frequency RR-Interval Oscillations in Humans

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Background—Survival of post–myocardial infarction patients is related inversely to their levels of very-low-frequency (0.003 to 0.03 Hz) RR-interval variability. The physiological basis for such oscillations is unclear. In our study, we used blocking drugs to evaluate potential contributions of sympathetic and vagal mechanisms and the renin-angiotensin-aldosterone system to very-low-frequency RR-interval variability in 10 young healthy subjects.

Methods and Results—We recorded RR intervals and arterial pressures during three separate sessions, with the patient in supine and 40 degree upright tilt positions, during 20-minute frequency (0.25 Hz) and tidal volume–controlled breathing after intravenous injections: saline (control), atenolol (0.2 mg/kg, β-adrenergic blockade), atropine sulfate (0.04 mg/kg, parasympathetic blockade), atenolol and atropine (complete autonomic blockade), and enalaprilat (0.02 mg/kg, ACE blockade). We integrated fast Fourier transform RR-interval spectral power at very low (0.003 to 0.03 Hz), low (0.05 to 0.15 Hz), and respiratory (0.2 to 0.3 Hz) frequencies. β-Adrenergic blockade had no significant effect on very-low- or low-frequency RR-interval power but increased respiratory frequency power 2-fold. ACE blockade had no significant effect on low or respiratory frequency RR-interval power but modestly ($\approx$21%) increased very-low-frequency power in the supine (but not upright tilt) position ($P<0.05$). The most profound effects were exerted by parasympathetic blockade: Atropine, given alone or with atenolol, abolished nearly all RR-interval variability and decreased very-low-frequency variability by 92%.

Conclusions—Although very-low-frequency heart period rhythms are influenced by the renin-angiotensin-aldosterone system, as low and respiratory frequency RR-interval rhythms, they depend primarily on the presence of parasympathetic outflow. Therefore the prognostic value of very-low-frequency heart period oscillations may derive from the fundamental importance of parasympathetic mechanisms in cardiovascular health. **(*Circulation*. 1998;98:547-555.)**

Key Words: receptors, adrenergic, beta ■ renin ■ heart rate ■ vagus nerve

Reductions of very-low-frequency RR-interval oscillations (with periods between 30 and 330 seconds or 0.03 to 0.003 Hz) are associated with increased risk for cardiac and dysrhythmic death[1,2] and possibly syncope.[3] Two mechanisms for these very slow heart period oscillations have been proposed: thermoregulation and the renin-angiotensin-aldosterone system. Because slow oscillations of peripheral vascular tone can be entrained by thermal stimuli at low frequencies,[4,5] Hyndman[6] and Kitney[7] suggested that they (and corresponding RR-interval rhythms) are in fact caused by thermoregulation.

A more recent suggestion is that very-low-frequency heart period oscillations reflect the influence of fluctuations of renin activity on arterial pressure. This hypothesis is supported by short-term studies conducted in resting, unanesthetized dogs[8] and long-term studies conducted in post–myocardial infarction patients,[9] which show that ACE blockade increases very-low-frequency heart period variability. These studies have at least two shortcomings. First, autonomic responses of healthy dogs may differ qualitatively from autonomic responses of patients.[10] Second, although Holter recordings permit analysis of very slow RR-interval fluctuations, they do not allow for control of common factors known to affect heart period variability such as posture, physical activity, breathing frequency, and tidal volume.[11,12] Moreover, the actual cascade of physiological events that generates very-low-frequency heart period variability has not been defined. For example, it is not known whether human arterial pressure oscillates at very low frequencies or if arterial pressure and heart period oscillate together, independent of respiration.

We assessed autonomic and renin-angiotensin-aldosterone system contributions to heart period and arterial pressure oscillations in healthy human subjects. Our paradigm enabled us to evaluate very-low-frequency oscillations with power spectrum analysis, without limitations inherent in Holter recordings. We recorded 20 minutes of beat-by-beat RR intervals and arterial pressures in healthy resting humans during controlled breathing in the supine and 40 degree upright tilt positions. We evaluated contributions of the

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autonomic nervous system with β-adrenergic blockade, cholinergic blockade, and complete autonomic blockade and contributions of the renin-angiotensin-aldosterone system with ACE blockade. Our results support a role for the renin-angiotensin-aldosterone system in very-low-frequency RR intervals but not in arterial pressure oscillations. More importantly, our data underscore the primacy of cardiac parasympathetic activity in generating short-term heart period oscillations and suggest that the prognostic value of heart period variability derives from the association between cardiac parasympathetic mechanisms and cardiovascular health.

Methods

Subjects

Ten healthy subjects (7 men and 3 women), 23 to 28 years of age, participated in this study. Volunteers were nonsmokers without histories of cardiovascular or other major diseases who were taking no cardioactive medications. Subjects refrained from alcohol or caffeine ingestion and strenuous physical activity for 24 hours preceding the study sessions. This research was approved by the human research committees of the Hunter Holmes McGuire Department of Veterans Affairs Medical Center and the Medical College of Virginia. All volunteers gave their written informed consent to participate.

Measurements and Protocol

Three sessions were conducted on separate days. For each session, drugs were administered through an antecubital vein catheter in a fixed order: (1) saline (control), atenolol (0.2 mg/kg, β-adrenergic blockade), and atropine sulfate (0.04 mg/kg, combined β-adrenergic and muscarinic cholinergic blockade); (2) saline, atropine, and atenolol; and (3) saline, enalaprilat (Merck, Sharp & Dohme, 0.02 mg/kg [slightly higher than the recommended clinical dose of 1.25 mg for a 70 kg patient], ACE blockade), and saline (placebo). Responses to each drug administration were assessed with the patient in the supine and 40 degree upright tilt position. (We used 40 degree tilt to increase sympathetic outflow because healthy young humans have low levels of sympathetic outflow in the supine position.[13]) Thus each of the 3 sessions comprised 6 trials (3 drug administrations in 2 positions). Session order and position order after drug administration were randomized, and subjects were not told which drugs they would be given.

During each trial, we recorded ECG lead II, beat-by-beat photoplethysmographic arterial pressure (Finapres, Ohmeda) in the finger, brachial arterial pressure (Dynamap, Critikon) once every 3 minutes, respiratory excursions (pneumobelt), breath-by-breath tidal volume (Fleisch pneumotachograph), and breath-by-breath end-tidal carbon dioxide concentration (infrared analyzer connected to a face mask with a 2-way respiratory valve). We recorded all signals continuously on FM tape for subsequent analog-to-digital conversion.

After catheter insertion, instrumentation, and instruction, subjects rested quietly for at least 10 minutes. Before each trial, subjects controlled their breathing frequency in response to an auditory signal at 0.25 Hz (15 breaths/min) to determine the most comfortable tidal volume. Subsequently, this inspired tidal volume was displayed on an oscilloscope to provide visual feedback so the subject could maintain a constant tidal volume. Controlled breathing was maintained for at least 20 minutes for each trial.

Immediately after the supine and tilt control trials, subjects were given either saline for autonomic studies or enalaprilat for renin-angiotensin-aldosterone system studies and then allowed to rest for an additional 25 minutes. This delay was included in the protocol to ensure full expression of the cardiovascular effects of enalaprilat. Although our dose reduces plasma angiotensin to less than one-tenth basal levels for up to 4 hours after intravenous infusion in young adults,[14] the peak cardiovascular effects of enalaprilat occur ≈1.5 to 2 hours after injection.[15,16] Therefore in addition to the 25 minutes of rest, only data from the third set of supine and tilt trials (which were at least 90 minutes after enalaprilat administration) were used to assess the effects of ACE blockade on cardiovascular variability. After administration of the autonomic blocking drugs, a 7-minute drug effect period was allowed before measurements during the supine and tilt trials.

Data Analysis and Statistics

All data were digitized at a rate of 500 Hz with commercial hardware and software (CODAS, Dataq Instruments). ECG R waves were identified to derive beat-by-beat RR intervals. Arterial pressure peaks and valleys were identified to derive beat-by-beat systolic and diastolic pressures. Mean and standard deviations for RR interval and systolic and diastolic pressures were calculated from the beat-by-beat values for each 20-minute trial.

Frequency domain analyses were performed on beat-by-beat RR intervals and systolic and diastolic pressures. We used a power spectrum analysis based on the Welch algorithm of averaging periodograms.[17] The 1200-second time series of beat-by-beat RR intervals and arterial pressures were interpolated by a cubic spline function at 4 Hz to obtain equidistant time intervals and then were divided into 5 equal overlapping segments. Each segment was detrended, Hanning filtered, and fast-Fourier transformed to its frequency representation squared. The periodograms were averaged to produce the spectrum estimate. Our estimation with the Welch method used 400-second segments to obtain estimates spaced at 0.0025 Hz, giving 12 estimates in the range of 0.0025 to 0.03 Hz and allowing detection of oscillations as slow as 0.0025 Hz. Areas under the power spectra in very low, low, and respiratory frequencies (defined as 0.003 to 0.03, 0.05 to 0.15, and 0.20 to 0.30 Hz) were integrated and used for statistical comparisons. Relative power (normalized units) was not calculated because we are skeptical of its validity as an accurate measure of cardiovascular variability.[18] (For example, in an earlier study, fixed-rate atrial pacing eliminated all RR-interval respiratory frequency spectral power in absolute values but did not alter spectral power in normalized units.[19]) To examine the strength of the relation between very-low-frequency RR-interval and systolic pressure variabilities, we derived the coherence estimate by cross-spectral analysis based on models described previously.[20,21] Although an estimate >0.5 has been used to signify that two cardiovascular signals covary significantly at a given frequency,[20] our spectral technique provided 9 degrees of freedom so that a minimum value of 0.58 was necessary to reject the null hypothesis that the coherence function was not different from 0 at a 0.05 significance level (see Appendix).

Effects of drugs and position on average RR intervals and arterial pressures for each session were evaluated with repeated-measures ANOVA and *t* tests with a Bonferroni post hoc correction to identify significant differences. Nonparametric statistics were used to examine effects on RR-interval and arterial pressure variabilities because spectral powers were not distributed normally, even after log transformation.[22] Spearman rank order correlations were calculated for control supine and tilt RR intervals and arterial pressures at very-low-frequency powers to assess consistency across sessions. A series of univariate sign-rank tests was used to assess drugs effects. Differences were considered significant at $P<0.05$. Measurements are reported as mean±SE.

Results

Respiration

Subjects controlled their respiration very well (average respiratory frequency for all trials: 0.24±0.002 Hz, 4.11 seconds per breath); they maintained average tidal volumes within 15% of target volumes in 176 of the 180 trials and within 20% of target volumes in the 4 remaining trials. Although end-tidal CO_2 concentrations tended to decrease from the beginning to the end of the 20-minute paced breathing trials, the average decline was <5%.

TABLE 1. Average RR Intervals and Arterial Pressures During Each Trial of the 3 Experimental Sessions

	RR Interval, ms		Systolic Pressure, mm Hg		Diastolic Pressure, mm Hg		Mean Pressure, mm Hg	
	Supine	Tilt	Supine	Tilt	Supine	Tilt	Supine	Tilt
Session 1								
Saline	955±50	809±39†	123±5	123±5	66±3	68±3	85±4	87±3
Atenolol	1067±46*	976±38*†	122±4	119±4	64±4	69±2	83±3	85±2
Double blockade	642±15*	641±14*	146±6*	131±5†	84±5*	79±3*	105±5*	97±3*†
Session 2								
Saline	924±51	779±36†	123±3	119±3	64±3	68±2	83±2	85±2
Atropine	588±13*	524±25*†	141±5*	127±3	85±4*	83±3*	103±4*	97±2*
Double blockade	695±22*	691±25*	138±6*	118±4†	85±4*	76±3*	103±4*	90±4†
Session 3								
Saline	973±56	808±30†	126±3	121±4	71±3	70±2	89±3	87±2
Enalaprilat	988±52	830±26†	128±4	128±4	69±3	74±3	88±3	85±2

*$P<0.05$ vs saline control; †$P<0.05$ vs supine, within drug. Values are mean±SEM.

Mean RR Interval and Arterial Pressures

Table 1 lists average RR intervals and arterial pressures for all trials. Upright tilt decreased RR intervals consistently ($P<0.05$) except after complete autonomic blockade. Upright tilt decreased systolic and mean arterial pressures significantly only after complete autonomic blockade ($P<0.05$). As expected, atenolol increased and atropine, with or without atenolol, decreased mean RR intervals (all $P<0.05$). Enalaprilat did not alter mean RR intervals and arterial pressures significantly. Atropine and atropine with atenolol increased average systolic pressure in the supine position and average diastolic and mean pressures in both the supine and tilted positions ($P<0.05$).

Figure 1 shows average RR-interval spectral power for three conditions for all subjects. The very sharp peaks at the imposed respiratory frequency (0.25 Hz) after saline and enalaprilat support the contention that our subjects controlled their breathing very well. The middle panel suggests that enalaprilat increased very-low-frequency RR-interval spectral power modestly, and the right panel indicates that atropine nearly abolished RR-interval spectral power at all frequencies. We discuss these changes below.

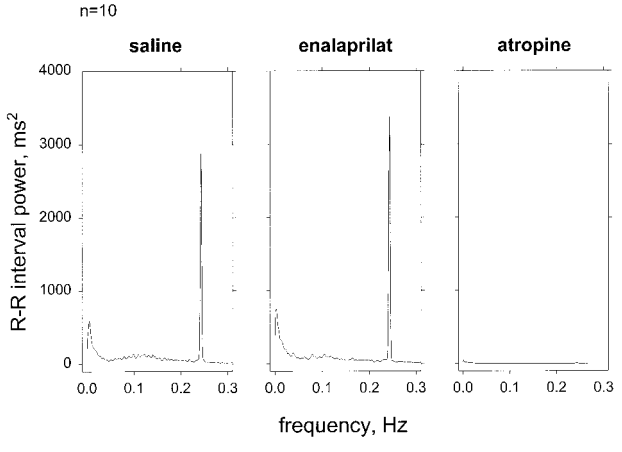

Figure 1. Average RR-interval spectral power for all subjects. The small increase of very-low-frequency spectral power after enalaprilat (middle panel, extreme left) was statistically significant.

Very-Low-Frequency Spectral Power

Figure 2, left, shows raw and filtered (low and high frequency cutoffs: 0.003 and 0.03 Hz) RR interval and systolic pressure recordings from one supine subject. The right panels of this figure show very-low-frequency spectral power calculated from the time series shown on the left. This and most other subjects had substantial RR-interval spectral power in the very-low-frequency range (averages for all subjects were supine: 23±3% and 40 degree tilt: 37±2% of total power). These measures tended to be consistent both within and across sessions ($r^2=0.67$, $P<0.08$; $r^2=0.72$, $P<0.05$). In contrast, although very-low-frequency spectral power accounted for a large portion of total systolic and diastolic pressure power (averages for all subjects were supine: 51±2% and 47±2%, tilt: 47±2% and 39±2% of total

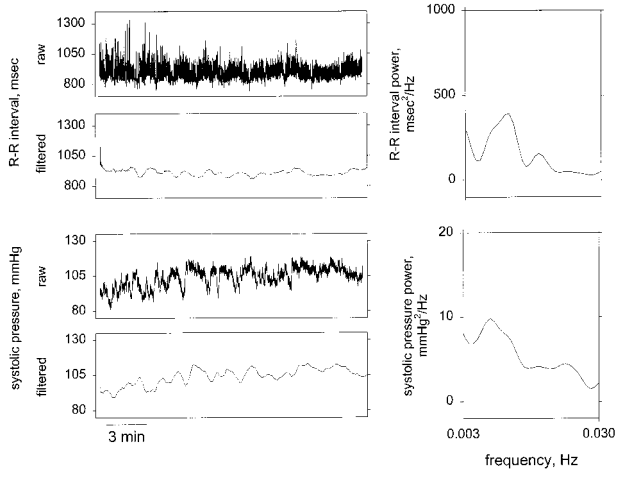

Figure 2. Twenty minutes of data from a representative supine subject. Beat-by-beat and 0.003 to 0.03 Hz filtered RR intervals and arterial pressure are shown on the left. Spectral power of the beat-by-beat data in the very-low-frequency range are shown on the right.

TABLE 2. Very-Low-Frequency (0.003 to 0.03 Hz) Variability in RR Interval and Arterial Pressures During Each Trial of the 3 Experimental Sessions

	RR Interval, ms^2		Systolic Pressure, mm Hg^2		Diastolic Pressure, mm Hg^2	
	Supine	Tilt	Supine	Tilt	Supine	Tilt
Session 1						
Saline	7702±3208	6905±1991	182±60	125±34	32±7	28±5
Atenolol	24 656±16 972	10 951±3941	109±25	155±41	28±5	38±9
Double blockade	102±34*	190±66*	55±11*	74±21*	27±4	34±7
Session 2						
Saline	10 157±4121	6505±1635	181±34	194±30	44±7	35±5
Atropine	349±127*	455±157*	106±21*	145±32	37±8	46±10
Double blockade	405±168*	531±302*	101±27*	119±32*	40±7	35±9
Session 3						
Saline	8308±2927	9671±2666	143±35	196±60	41±6	39±9
Enalaprilat	10 041±3012*	11 634±3574	128±16	160±37	39±8	50±9

*$P<0.05$ vs saline control. Values are mean±SEM.

power), very-low-frequency arterial pressure powers were consistent only within sessions (systolic $r^2=0.82$, diastolic $r^2=0.61$; $P<0.05$) and not across sessions ($r^2=0.44$ and 0.23, $P>0.20$). Table 2 lists average integrated spectral power at very low frequencies before and after administration of blocking drugs for all subjects.

Figure 3 shows average (±SE) coherence between systolic pressure and RR intervals over the very-low-frequency range for all subjects for one trial. Average coherence was <0.58 over almost all of the very-low-frequency range. The low reproducibility of arterial pressure spectral power, discussed above, may reflect the lack of coherence between RR intervals and systolic pressure at very low frequencies. Coherence in the very-low-frequency range was quite variable both within and among subjects. Six subjects had significant coherence between RR intervals and systolic pressure in at least 1 of the 6 control trials (supine and tilt); no control trial demonstrated coherence >0.58 in more than 2 subjects. Thus we found no consistent relation between RR intervals and systolic arterial pressure in the very-low-frequency range.

Figure 4 shows changes of very-low-frequency RR-interval spectral power before and after blocking drugs for all subjects. Although atenolol (top panel) appeared to increase very-low-frequency RR-interval spectral power, the range of changes was large, and the spectral powers in the supine and tilted positions were not statistically different from those measured after saline administration ($P=0.16$). [The apparent increase of RR-interval spectral power after atenolol (top panel, left) reflected an inordinately large increase in 1

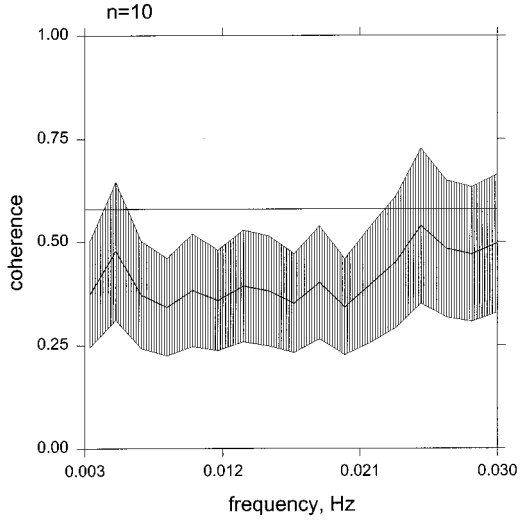

Figure 3. Mean and standard error of the coherence between systolic pressure and RR interval across the very-low-frequency range. When the coherence exceeded 0.5 within a frequency range, the two signals were considered to covary significantly at that frequency.

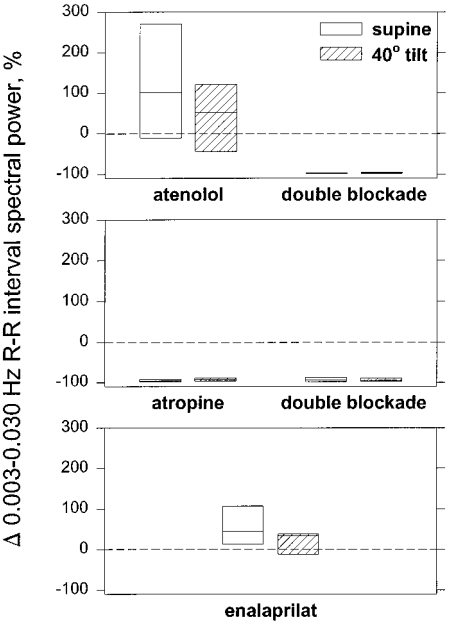

Figure 4. Change from control for very-low-frequency RR-interval power (median, 25th and 75th percentiles) for all blockade conditions. Double blockade and atropine decreased power; enalaprilat increased power in the supine position only ($P<0.05$).

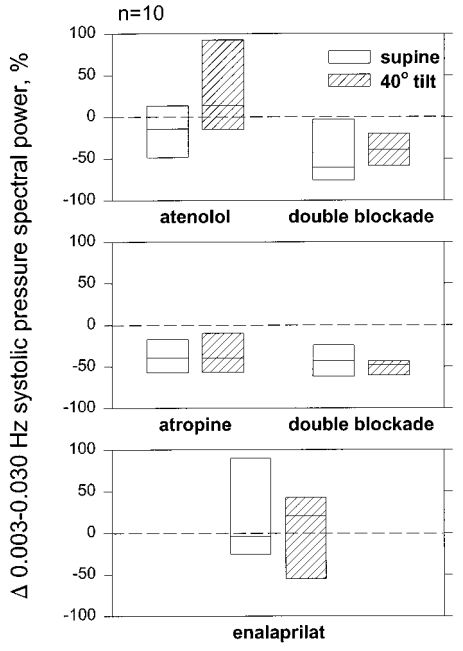

Figure 5. Change from control for very-low-frequency systolic pressure power (median, 25th and 75th percentiles) for each blockade condition in supine and tilted positions. Atropine and double blockade decreased power ($P<0.05$, except tilt after atropine $P=0.11$).

subject. Without this subject (whom we had no other reason to exclude), the median increase of very-low-frequency RR-interval spectral power after enalaprilat was 66% ($P=0.30$).] Enalaprilat (bottom panel) exerted only a modest effect on very-low-frequency RR-interval spectral power [the increase averaged 21% in the supine position], which was, nonetheless, statistically significant ($P<0.05$). Increases of very-low-frequency RR-interval spectral power after enalaprilat in the tilted position were not statistically significant. The most striking changes were exerted by atropine (top two panels, right). Atropine with or without atenolol nearly eliminated very-low-frequency RR-interval spectral power in both supine and tilted positions (decreases averaged 92% to 99% of control).

Figure 5 shows and Table 2 lists average changes of very-low-frequency systolic pressure spectral power before and after blocking drugs for all subjects. Neither atenolol nor enalaprilat significantly altered very-low-frequency systolic pressure spectral power. In contrast, atropine decreased very-low-frequency systolic pressure power (all $P<0.05$ except atropine in the tilt position, $P=0.11$ saline versus atropine). Very-low-frequency diastolic pressure power (not shown) was not affected significantly by any blockade.

Low and Respiratory Frequency Spectral Power

Tables 3 and 4 list mean low and respiratory frequency RR-interval and arterial pressure spectral power for all trials. Atenolol increased RR-interval spectral power only at the respiratory frequency ($P<0.05$). Enalaprilat had no effect on either low or respiratory frequency RR-interval spectral power. As Figure 1 indicates, atropine with or without atenolol nearly abolished RR-interval spectral power in all frequency bands. Upright tilt significantly reduced low-frequency RR-interval spectral power in only 1 of the 3 saline trials ($P<0.05$). Upright tilt after atropine with or without atenolol led to a small but significant ($P<0.05$) increase of low-frequency RR-interval spectral power. Upright tilt decreased respiratory frequency RR-interval spectral power except after parasympathetic or complete autonomic blockade ($P<0.05$).

Atropine with or without atenolol reduced low and respiratory frequency systolic pressure power in the supine position. Atropine reduced low-frequency diastolic pressure spectral power in the supine position. Combined atropine and atenolol reduced respiratory frequency diastolic pressure power in the supine position, and atropine alone increased respiratory frequency diastolic pressure spectral power in the tilted position. ACE blockade did not affect low or respiratory frequency arterial pressure spectral power significantly in either position.

TABLE 3. Low-Frequency (0.05 to 0.15 Hz) Variability in RR Interval and Arterial Pressures During Each Trial of the 3 Experimental Sessions

	RR Interval, ms^2		Systolic Pressure, mm Hg^2		Diastolic Pressure, mm Hg^2	
	Supine	Tilt	Supine	Tilt	Supine	Tilt
Session 1						
Saline	7335±1451	6041±1245	42±11	52±11	16±3	27±5
Atenolol	21 526±12 879	7361±1723	39±12	51±13	24±9	28±9
Double blockade	13±3*	95±47*†	13±3*	56±22	10±5	38±12
Session 2						
Saline	13 414±3650	6565±1847†	64±15	85±14	28±6	38±7
Atropine	39±12*	246±128*†	24±6*	123±36	12±3*	65±18
Double blockade	75±28*	257±164*	26±11*	79±17	12±4	44±12
Session 3						
Saline	11 343±3278	8958±2659	38±7	69±16	21±4	32±6
Enalaprilat	10 218±2086	8707±2100	33±5	59±13	19±4	35±6

*$P<0.05$ vs saline control; †$P<0.05$ vs supine, within drug. Values are mean±SEM.

TABLE 4. Respiratory Frequency (0.2 to 0.3 Hz) Variability in RR Interval and Arterial Pressures During Each Trial of the 3 Experimental Sessions

	RR Interval, ms^2		Systolic Pressure, mm Hg^2		Diastolic Pressure, mm Hg^2	
	Supine	Tilt	Supine	Tilt	Supine	Tilt
Session 1						
Saline	18 927±6408	4315±1495†	38±8	59±10	7±2	10±2
Atenolol	37 928±12 762*	10 956±4150*†	48±11	82±16	10±4	5±1
Double blockade	48±13*	134±108*	18±3*	71±12	4±1	16±6
Session 2						
Saline	21 172±4791	4015±1587†	58±12	77±13	11±3	9±2
Atropine	40±17*	14±3*	25±2*	95±18	11±3	37±7*
Double blockade	77±27*	110±65*	24±1*	91±10	5±1*	12±3
Session 3						
Saline	16 877±4484	3297±1042†	38±6	49±5	9±3	6±1
Enalaprilat	19 261±5672	4121±853†	44±6	64±13	8±1	9±4

*$P<0.05$ vs saline control; †$P<0.05$ vs supine, within drug. Values are mean±SEM.

Discussion

We studied 10 healthy young adult volunteers in the supine and 40 degree upright tilted positions, before and after autonomic or ACE blockade, to explore mechanisms responsible for very-low-frequency cardiovascular rhythms. Our measurements were not confounded by changes of respiration, posture, or physical activity, which markedly alter RR-interval and arterial pressure variability, and our recording periods were long enough to permit us to draw meaningful inferences regarding very-low-frequency rhythms. Our study supports 3 primary conclusions. First, although healthy humans have substantial RR-interval and arterial pressure spectral power in the 0.003 to 0.03 Hz range, there appears to be no consistent linkage between the two rhythms. Thus very-low-frequency RR-interval rhythms cannot be explained simply in terms of baroreflex mechanisms. Second, as others before us,[9] we document a contribution from the renin-angiotensin-aldosterone system to very-low-frequency cardiovascular rhythms; however, our results indicate that this contribution is small and that it involves RR intervals but not arterial pressures. Third and most important, our study shows that contributions from parasympathetic activity dominate very low (as well as higher) frequency RR-interval rhythms.

Thermoregulatory Mechanisms

Two primary (not mutually exclusive) mechanisms have been proposed to explain very-low-frequency RR-interval variability. The mechanism proposed first was that very-low-frequency cardiovascular rhythms reflect thermoregulation. In our view, published evidence supports this possibility, but only indirectly. It is clear that cutaneous blood flow oscillates slowly[23] and that cutaneous blood flow[4] and RR-interval[5] oscillations can be entrained by externally applied oscillatory temperature changes. However, a direct link between cutaneous thermoregulatory rhythms and cardiovascular rhythms has not been established; to our knowledge, no one has actually documented changes of body core temperature at very low frequencies and shown with coherence analysis that cutaneous blood flow, arterial pressures, and RR intervals fluctuate together. Thus the linked hypotheses that thermoregulatory skin blood flow rhythms translate into arterial pressure rhythms and that arterial pressure rhythms translate into baroreflex-mediated RR-interval fluctuations[7] have not been validated. We did not measure body core temperature in our study; however, we did measure arterial pressure and RR intervals and failed to find the baroreflex linkage that is a critical element of the thermoregulatory hypothesis.

Renin-Angiotensin-Aldosterone Mechanisms

The mechanism proposed second was that very-low-frequency RR-interval rhythms reflect influences of the renin-angiotensin-aldosterone system. As mentioned, Akselrod and coworkers[8] reported that ACE blockade increases very-low-frequency RR-interval spectral power. However, data from other studies, also conducted in conscious dogs, challenge Akselrod and colleagues' conclusions (which were based on results obtained during 5-minute recordings from only 3 dogs). Brown et al[24] and Rimoldi et al[25] reported no change or an actual reduction of RR-interval (and systolic pressure) spectral power after ACE blockade. Our study confirms the findings of Akselrod et al in the sense that we found that ACE blockade increases very-low-frequency RR-interval spectral power (but modestly, not dramatically).

Before our study, most published data on very-low-frequency RR rhythms in humans came from 24-hour Holter monitor recordings.[1,2,9,26,27] Although Holter monitor recordings provide a sufficiently long data collection period to document fluctuations occurring as slowly as only once every 5.5 minutes (0.003 Hz.), Holter recordings are not controlled for common factors known to affect RR-interval variability, including posture, activity, breathing frequency, and tidal volume.[11,12] Holter recordings obtained in post–myocardial infarction patients[9,28] and in congestive heart failure patients[29] have shown that ACE blockade increases both frequency and time domain measures of very-low-frequency RR-interval variability. However, these findings have not been replicated

with Holter recordings in healthy subjects.[27] This discrepancy may reflect a greater physiological role of the angiotensin system in patients with cardiovascular disease. Our protocol differs from the earlier studies in patients[9,28,29] in that our subjects were young and healthy and from the Holter studies in healthy subjects[27] in that our subjects controlled their respiratory frequency and tidal volume and we controlled body position. Nonetheless, our findings support a role for the renin-angiotensin-aldosterone system in very-low-frequency RR-interval fluctuations and provide new information regarding autonomic mechanisms.

Our results do not indicate explicitly how ACE blockade increases very-low-frequency RR-interval spectral power; we do not know if this observation reflects episodic increases of plasma renin activity, potentiation of bradykinin (which accounts for a portion of the hypotensive effects of ACE inhibitors[30]), or modulation of some other neurohumoral influence that fluctuates at very low frequencies. We are intrigued by the observation that ACE blockade increased RR-interval spectral power in the supine but not the upright tilted position. Although we cannot exclude a β-statistical error (that we erred by studying too few subjects), our observation may have a physiological basis: Increases of angiotensin II levels occur episodically, as arterial pressure fluctuates above and below a threshold.[31] During upright tilt, arterial baroreceptor input may remain consistently below the threshold for increases in plasma renin activity. Therefore although absolute levels of plasma renin activity are increased in the upright position, their fluctuations might be reduced.

Serial measurements of plasma renin activity have defined the capacity of the kidney to modulate renin activity in response to sinusoidal variations in renal arterial pressure at 0.002 Hz.[32] However, even if fluctuations of plasma renin activity occur within the very-low-frequency range, they may not provoke systemic hemodynamic changes at these frequencies. A study published by Cowley et al,[33] conducted in dogs, suggests that responses to changes of plasma renin activity levels occur too slowly to influence very-low-frequency events. Arterial pressure increases provoked by step reductions of renal artery pressure occur slowly, over $\approx$15 to 30 minutes.

A second explanation for our observations is that steady plasma renin activity levels modulate other neurohumoral mechanisms that fluctuate at very low frequencies. Akselrod and coworkers[8] speculated that chronic levels of renin activity and angiotensin dampen fluctuations of peripheral vasomotor tone and that ACE blockade increases vasomotor tone fluctuations and (presumably by an arterial baroreflex mechanism) corresponding RR-interval fluctuations. Our study does not support a baroreflex mechanism; we found no increase of arterial pressure spectral power after enalaprilat (Figure 5) and no significant coherence ($>$0.58; see Appendix) between RR intervals and arterial pressure at very low frequencies (Figure 3). Alternatively, angiotensin blockade may enhance cardiac vagal outflow[34,35] and thus increase cardiac vagal oscillations at very low frequencies (see discussion below).

Vagal Mechanisms

Parasympathetic blockade exerted the most dramatic effect of all the pharmacological interventions we used. Atropine nearly abolished very-low-frequency RR-interval power (Figure 1 and Table 2). Atropine also nearly abolished low and respiratory frequency power, as described previously.[33–35] Our study suggests that the parasympathetic nervous system is prepotent in the generation of all the RR-interval oscillations we studied, including those occurring at very low, low, and respiratory frequencies. We advance a simple, economical explanation for our findings—that efferent vagus nerve traffic to the human heart fluctuates over very low to respiratory frequencies and that large-dose atropine blocks sinoatrial node responses to those fluctuations. Atropine does not alter RR intervals when vagus nerve traffic is absent.[36] A corollary of this is that the adverse prognostic significance of low levels of very-low-frequency RR-interval fluctuations in postinfarction patients[1,2] is tied to reductions of efferent vagal-cardiac nerve traffic.

Limitations

We believed that it was important for our subjects to control their breathing so they would avoid the huge (10-fold) variations of RR-interval spectral power that result from variations of breathing frequency.[12] Although our subjects maintained constant respiratory rates and tidal volumes longer (20 minutes) than those of any other study to date, our study would have been strengthened if our subjects had maintained constant breathing for even longer periods. However, our confidence in our power estimates increases as the frequency of interest increases. For example, a 20-minute breathing period includes 3.6 cycles of 0.003 Hz but 12 cycles of 0.01 Hz. (More than half of the RR variability within the very-low-frequency range was $>$0.01 Hz in 70% of the trials.) We used 20-minute periods because pilot studies showed that even dedicated volunteers have difficulty maintaining constant breathing continuously for $>$20 minutes and because our protocol required 6 trials for each experimental session. Because our measurement periods were only 20 minutes, we can say nothing about ultralow-frequency rhythms. This may not pose a problem, however, because a study of Bigger et al[1] showed that very-low-frequency RR-interval spectral power has major prognostic significance in postinfarction patients, even when it is calculated over epochs lasting $<$20 minutes.

The validity of our conclusion that very-low-frequency RR-interval fluctuations in humans are not mediated by baroreflex mechanisms hinges on the fact that our estimates of coherence between arterial pressure and RR intervals $<$0.58 are not statistically significant (see Appendix). Although our subjects controlled such input variables as respiration and physical activity, we cannot exclude the possibility that arterial pressure and RR intervals are related nonlinearly at very low frequencies. Moreover, studies in cats[36] and dogs[37] document baroreflex influences at very low frequencies.

Finally, the effects of our pharmacological interventions may have been circumscribed by the dosages used. For example, we gave the hydrophilic β-adrenergic–blocking drug atenolol in a dose that was worked out for the lipophilic

β-adrenergic–blocking drug propranolol.[38] However, we propose that our atenolol dose is justified on three counts: Atenolol enters the central nervous system[5]; atenolol and metoprolol, another lipophilic β-adrenergic–blocking drug, enhance 24-hour RR-interval spectral power equally[39]; and none of our conclusions are contingent on β-adrenergic blockade being "complete." It may also be argued that our (slightly more than) clinical dose of enalaprilat produced no profound physiological effects suggestive of ACE inhibition. Yet it may not be surprising that our young (23 to 28 years old), normotensive (Finapres-derived systolic pressure of 126 mm Hg) subjects demonstrated no profound changes in average RR interval or arterial pressures with enalaprilat. Our dose of enalaprilat does not alter RR interval and reduces arterial pressure greatest in individuals with highest basal pressure,[14] describing a relation between basal systolic pressure and pressure reduction with an intercept ≈120 mm Hg. Last, it should be noted that physiological effects of clinical doses of enalaprilat and atenolol may not be absolutely analogous to those of complete blocking doses of atropine.

In summary, our study provides support for previous observations that the renin-angiotensin-aldosterone system influences very-low-frequency RR-interval variability. In addition, our study addresses the role of the autonomic nervous system in the generation of these RR-interval oscillations: We found that as with respiratory and low-frequency RR-interval variabilities, very-low-frequency RR-interval oscillations are very much dependent on parasympathetic tone. Therefore the prognostic value of very-low-frequency heart period oscillations may derive from the fundamental importance of parasympathetic mechanisms in cardiovascular health.

Appendix

The coherence of time series $x(t)$ and $y(t)$ measures the variance of y linearly predictable from x at each frequency f.[40] Coherence serves as the frequency domain analog of r^2, the coefficient of determination. Like r^2, its value lies between 0 and 1, with values near 1 indicating a strong linear relation between the two series. Coherence is defined as the ratio of the squared covariance of the 2 series to the product of their individual variances. If P_{xx} and P_{yy} denote the autospectra of x and y, and P_{xy} denotes their cross-spectrum, then coherence $\gamma^2(f)$ is given at each frequency f by

$$\gamma^2(f)=\frac{|P_{xy}(f)|^2}{P_{xx}(f)\,P_{yy}(f)} \quad (1)$$

De Boer et al[20] proposed that coherence level indicates the strength of the linear association and provides a gauge of variability in phase estimates between two cardiovascular variables. After the work of de Boer et al,[20] it has become common to claim a significant linear relation between two cardiovascular time series when coherence values exceed 0.5. Although 0.5 was the correct threshold for significance based on the parameters of de Boer and colleagues' estimation procedure, other parameters or estimation procedures will in general determine a different threshold necessary to accept the hypothesis that coherence significantly exceeds 0. These parameters effect the degrees of freedom (d) of the estimate which, with a significance level α, can be used to determine the minimal coherence (γ^2_{min}) to reject the null hypothesis with an F test by

$$\gamma^2_{min}=\frac{2F_{2,d-2}(\alpha)}{d-2+2F_{2,d-2}(\alpha)} \quad (2)$$

The degrees of freedom derive from the relations between sample size, segment or window length, and window shape. Sampling a data series of duration T seconds at an interval of Δt seconds, or a sampling rate of $f_s=1/\Delta t$ Hz, produces $N=T/\Delta t$ samples. The averaged periodogram method of power spectrum estimation (eg, Welch[17]) divides these N samples into segments of length L (a duration of $L\Delta t$), whereas the correlogram method (eg, Blackman and Tukey[41]) applies a single window of length L. The optimal length depends on the desired balance between low variance (obtained by small L) and high resolution (obtained by large L) but must contain at least one full cycle of the lowest frequency oscillation of interest; for example, at least 300 seconds (1/0.0033 Hz) is required for the very-low-frequency band, 0.0033 to 0.03 Hz. The window used to smooth the spectral estimate determines a multiplicative constant that increases the degrees of freedom.[40] Nonetheless, the degrees of freedom are roughly proportional to N/L. For a sufficiently large N, the estimated coherence can be approximated by a χ^2 distribution.[17,40] Thus equation 2 merely applies an F test with 2 and d-2 degrees of freedom.[40]

As an example, we used the periodogram method of Welch[17] to compute our estimates of spectra and calculated coherence to investigate whether the variance of very-low-frequency oscillations in RR interval and systolic pressure had a significant linear relation. Our analysis divided $N=4800$ samples into segments of length $L=1600$, which overlapped by half, were detrended, and were multiplied by a Hanning window (constant=2.67). Overlapping segments decreases the variance of the estimated spectrum and increases the degrees of freedom[17] such that in this case the window constant effectively becomes 2.83. Thus our estimates have $d=2.83N/L=8.49$, or 9 degrees of freedom. For $d=9$ and $\alpha=0.05$, we have $F_{2,7}(0.05)=4.74$. Using equation 2, we can reject the null hypothesis that coherence equals 0 when our estimate exceeds 0.58 at the $\alpha=0.05$ level. At the $\alpha=0.10$ and $\alpha=0.01$ levels, minimal coherence values are 0.48 and 0.73.

Although minimal coherence is specific to the spectral technique, coherence estimated by other techniques (eg, autoregressive models) can be similarly analyzed for significance.

However, the cutoff for minimal coherence should not be applied indiscriminately. The level of 0.5 can be defended because it suggests a relation between two signals based on 50% shared variance, whereas an F test with sufficient degrees of freedom can indicate that a low shared variance (ie, low coherence value) significantly differs from 0. Nonetheless, it is important to note that spectral techniques with few degrees of freedom can produce high coherence values that do not significantly differ from 0. Thus meaningful interpretation of coherence should consider the level of confidence in the estimate.

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