Heart rate variability and sympathovagal balance: pharmacological validation

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Rationale. We validated heart rate (HR) and six time and six frequency domain measures of heart rate variability (HRV) as estimators of autonomic outflow in 44 young healthy male subjects. Gold standards for autonomic outflow were the Rosenblueth-Simeone factors m (sympathetic tone) and n (vagal tone), and the sympathovagal balance m·n, determined by two-stage complete autonomic blockade.

Methods. Rank correlations were computed between HR and the HRV measures obtained before autonomic blockade, and m, n and m·n. Also, the maximal mean performances (averaged sensitivity and specificity) for HR and HRV as discriminators between low and high values of m, n or m·n were computed.

Results. The spectral HRV measures showed less good correlations and performances than the time domain HRV measures. Correlations with sympathetic tone were all below 0.31. Respiratory sinus arrhythmia during 15 cycles/min metronome breathing was superior in estimating vagal tone and sympathovagal balance (correlations -0.71/-0.73; both performances 0.82), heart rate scored similarly for assessing the sympathovagal balance (correlation 0.71; performance 0.82).

Conclusions. It does not appear justified to evaluate HR or HRV in terms of sympathetic tone, vagal tone, or sympathovagal balance. HR and HRV are specifically weak in assessing sympathetic tone. Respiratory sinus arrhythmia during 15 cycles/min metronome breathing is superior in assessing vagal tone. Current HRV analysis techniques offer no advantages compared with HR in assessing the sympathovagal balance. (*Neth Heart J* 2003;11: 250-9.)

Key words: atropine, metoprolol, heart rate variability, Rosenblueth-Simeone model, sympathovagal balance

The sinoatrial node is subject to influences from the autonomic nervous system which modulate its discharge rate. In autonomic states between rest and light exercise, the actual heart rate (HR) is determined by simultaneous sympathetic acceleration and vagal deceleration. Rosenblueth and Simeone[1] demonstrated that the combined sympathetic and vagal influences on sinus node automaticity can be expressed by the product of the separate sympathetic and vagal effects and the intrinsic heart rate: $HR=m \cdot n \cdot HR_0$ where m is a factor representing sympathetic acceleration ($m \geq 1$), n a factor representing vagal deceleration ($n \leq 1$), and HR_0 the intrinsic heart rate. The product m·n ($= HR/HR_0$) can be regarded as the sympathovagal balance.[2,3] This sympathovagal balance is ≤ 1 or ≥ 1 under conditions of vagal or sympathetic predominance, respectively.

Simultaneous pharmacological blocking of the adrenergic and cholinergic influences to the heart is needed to determine HR_0. If this is accomplished in two stages, the sympathetic and vagal factors m and n can be computed from the HR changes due to initial adrenergic and subsequent cholinergic blocking, respectively.[3,4] A noninvasive procedure would, however, be preferable not only for general reasons but also because venous cannulation, as required for pharmacological blocking, has autonomic consequences.[5] Heart rate variability (HRV) analysis has been postulated to offer this noninvasive alternative. HRV is caused by beat-to-beat fluctuations in sympathetic and vagal outflow. The sinus node functions as a low-pass filter for sympathetic and vagal stimuli. HRV interpretation is based on a

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lower cut-off frequency for sympathetic than for vagal fluctuations.[6,7] Thus, the sinus node can follow faster vagal fluctuations than sympathetic fluctuations. Rapid HRV, >0.15 Hz, therefore constitutes solid evidence for rapid fluctuations in vagal outflow. At the same time, rapid sympathetic fluctuations may occur, but these remain hidden when the cardiac rhythm is observed because of the lower cut-off frequency for sympathetic stimuli. Slower HRV, <0.15 Hz, may be caused by a mixture of sympathetic and vagal fluctuations.[8-10]

Several attempts have been made to assess sympathetic and vagal influences.[11] To estimate the amount of vagal involvement in HRV, mathematical techniques have been developed that assess preference of fast over slow HRV, or which determine the fraction of fast and total HRV or the ratio of fast and slow HRV. It was hoped that such measures would assess sympathetic tone, vagal tone, or the sympathovagal balance. However, the hypotheses underlying these efforts are open to criticism.[12] Firstly, HRV is not exclusively mediated by fluctuations in sympathetic and vagal outflow, but is also mechanically induced by respiration.[13] Secondly, slow HRV is mediated by an unknown mixture of sympathetic and vagal fluctuations.[10] Thirdly, HRV is caused by fluctuations in sympathetic and vagal outflow, and not by the average outflow (i.e. tone) itself, and there is no theory or model available that explains the relation between fluctuations in autonomic outflow and the average outflow.

The current study was designed to validate HR and HRV measures with respect to the Rosenblueth-Simeone model in healthy young subjects, by comparison of HR and HRV measures, determined in control conditions, with values of m, n, and m·n, obtained by consecutive two-stage pharmacological autonomic blockade.

Methods

Subjects

The study protocol was approved by the institution's Ethics Committee. Fifty subjects were recruited by advertisements in the local university press and agreed to participate after oral and written explanation of the study had been given. Heterogeneity in fitness level was purposely introduced by explicitly recruiting subjects who were active in leisure sports as well as subjects who had a sedentary lifestyle. All study subjects were apparently healthy, as evidenced by their medical history, a physical examination and a 12-lead ECG. They were not on any medications, and had no history of cardiovascular, pulmonary or other systemic disease. As a measure of cardiopulmonary fitness, the maximal oxygen uptake (VO_2 max) was determined by a bicycle exercise test (Bruce protocol, initial load 40 Watts, with 20 Watt increments every minute).

Measurement session

The invasive measurements were done on a separate day. The session was held in a quiet room (temperature 22°C), between 8.30 and 11.00 hours, after a light breakfast. The subjects were instructed to abstain from beverages containing caffeine and from alcohol from 20.00 hours the preceding day. Smoking was not allowed on the morning of the measurements.[14] During sessions the subjects were not allowed to speak; they were entertained with a videotape to prevent mental stress or falling asleep.

Throughout the session, a two-lead ECG and a single-lead respiration signal (thoracic impedance) was recorded on a Marquette Holter recorder (Marquette Electronics Inc., Milwaukee, WI, US). For monitoring purposes, the ECG was also continuously visualised and the arterial blood pressure was intermittently measured at the left upper arm (Accutorr 3, Datascope Corp., Montvale, NJ, US).

The measurement protocol was as follows (figure 1). Initially, the subjects rested for 25 minutes in the supine position. Then, a cannula (Venflon, BOC Ohmeda AB, Helsingborg, Sweden) was placed in a vein in the left forearm for the administration of the autonomic blocking agents. Subsequently, a two-minute handgrip at 30% of maximal force was performed; this was followed by a second stabilisation period of ten minutes (the handgrip data were obtained for the purpose of another study). Next, the subjects were instructed to breathe for two minutes at a rate of 6 cycles/min (0.10 Hz), and then, after a one-minute recovery period, for two minutes at a rate of 15 cycles/min (0.25 Hz). These two episodes were used to determine respiratory sinus arrhythmia during metronome breathing. They were then observed during a six-minute episode to measure the control resting HR, respiratory sinus arrhythmia during free respiration, and the other time and frequency domain HRV parameters.

Subsequently, functionally complete adrenergic blockade[3,15] was accomplished by four equal doses (4x0.05 mg/kg) of metoprolol, given every three minutes; the subjects thus received a total dose of 0.2 mg/kg of metoprolol. After metoprolol infusion, metronome respiration at 6 and 15 cycles/min was repeated for the purpose of another study. Thereafter, the HR under complete adrenergic blockade was measured in a six-minute episode.

Then, functionally complete cholinergic blockade[3,15] was accomplished by four equal doses (4x0.01 mg/kg) of atropine, given every three minutes.[4] The subjects thus received a total dose of 0.04 mg/kg atropine. Finally, the intrinsic HR[16] was measured during a last six-minute episode.

Data analysis

The ECGs were analysed with a Marquette Series 8000 Holter Analyser (Marquette Electronics Inc., Wilwaukee, WI, US). This Holter Analyser has a sampling rate of 128/sec (time resolution about 8 msec). A full dis-

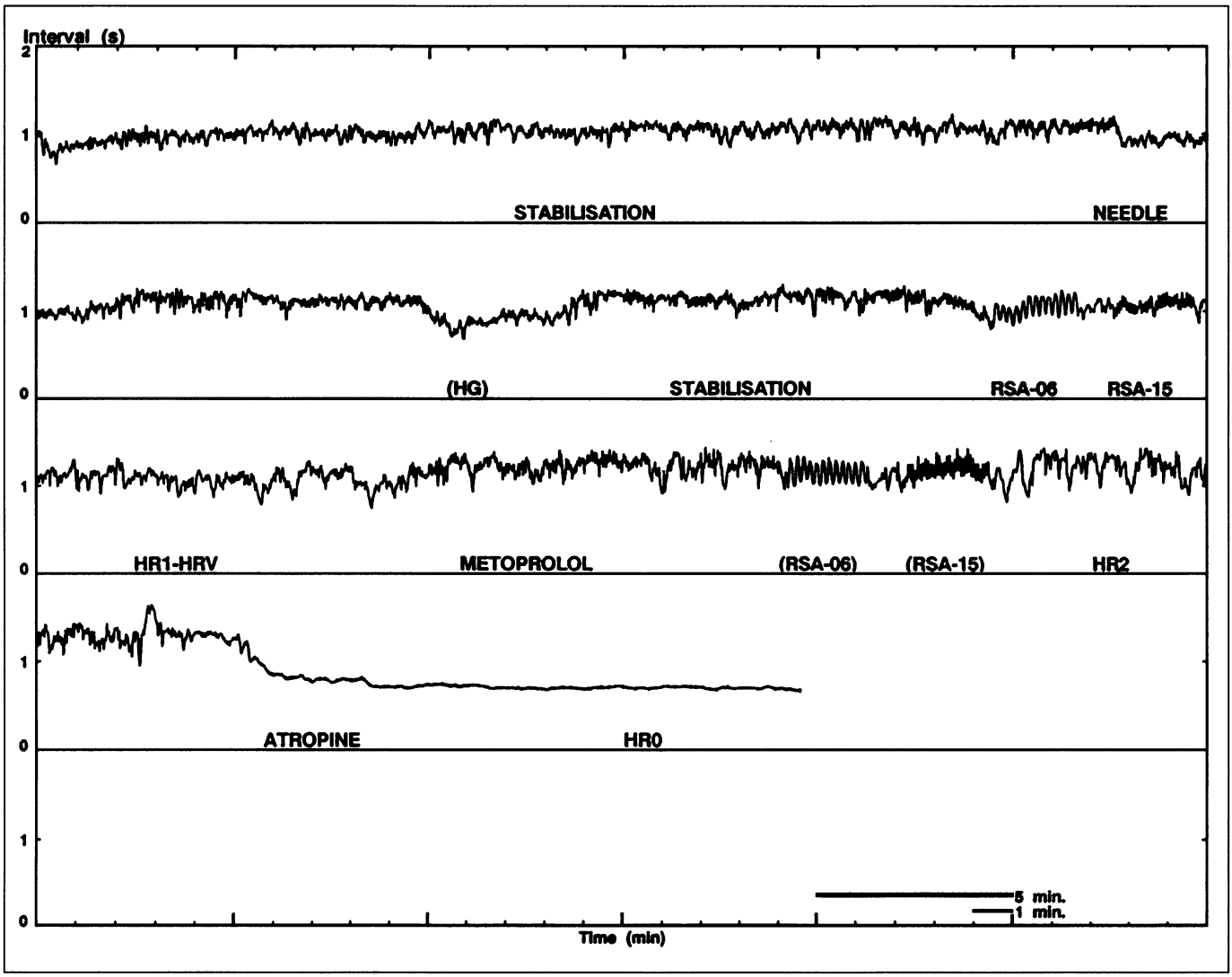

Figure 1. Interval tachogram (5 consecutive 30-minute tracings) recorded during the measurement session of subject (#36). Upper tracing: initial 25-minute episode of supine stabilisation, after which a cannula is inserted. Second tracing: following needle insertion, instructions for handgrip are given, handgrip (HG) at 30% of maximal force is applied for two minutes, then there is again a stabilisation episode, followed by two-minute episodes of 6 cycles/min and 15 cycles/min metronome breathing (RSA_{06} and RSA_{15}), separated by a one-minute recovery period. Third tracing: just before adrenergic blockade with metoprolol there is a six-minute episode in which HR1 and HRV are measured, after complete adrenergic blockade there are again 6 and 15 cycles/min breathing episodes, followed by a six-minute episode of rest (HR2), preceding blockade with atropine. The tachogram clearly shows that handgrip accelerates HR, the 6 and 15 cycles/min breathing episodes show HRV in which the respiratory frequency predominates, adrenergic blockade with metoprolol decreases HR, atropine increases HR (HR_0), and HRV disappears.

closure printout of the respiration signal was used to assess the spontaneous breathing frequency and to verify compliance with metronome breathing. Accurate determination of the onset of the QRS complexes was accomplished by an extensive review and edit procedure, also using the CCTOC programme of the Marquette 8000 Holter research software modules. The resulting inter-beat-interval series were further analysed with a personal computer.

HR values were computed in the following six-minute episodes: before metoprolol (HR_1), before atropine (HR_2), and after complete autonomic blockade (HR_0). The sympathetic factor m was calculated by dividing HR_1 by HR_2, and the vagal factor n was calculated by dividing HR_2 by HR_0. Then, the sympathovagal balance was computed as m·n.

Time and frequency domain values of HRV, including respiratory sinus arrhythmia (RSA) during free respiration, were computed in the six-minute episode before metoprolol. RSA measures during fixed respiration were computed in the preceding metronome breathing episodes. The following time domain

measures were calculated: 1) standard deviation of inter-beat-intervals (SDNN); 2) coefficient of variation (CV), obtained by division of SDNN by the average inter-beat-interval and expressed as a percentage; 3) percentage of successive inter-beat-intervals differing more than 50 ms (pNN50); 4) RSA during 6 cycles/min metronome breathing (RSA_{06}); 5) RSA during 15 cycles/min metronome breathing (RSA_{15}); and 6) RSA during free respiration (RSA_{fr}).

Values of RSA were computed using the following algorithm. The episode in which RSA had to be assessed was divided into segments with a duration identical to the respiration period. Within each segment, the minimal and maximal inter-beat-intervals were determined. If the distance in time between the occurrence of these extrema was within one second from half the duration of the breathing cycle, the difference of the maximal and minimal inter-beat-intervals was accepted as a valid measurement for RSA estimation. For the episode RSA value, all accepted segment RSA values were averaged.

Frequency domain HRV measures were computed as follows. First, the means and variances of the inter-beat-intervals in twelve 30-second subsegments were computed, and episode stationarity was verified by applying reverse arrangement tests at the 10% level to these means and variances.[17] Spectral analysis was carried out according to the following algorithm.[2] First, the intervals were normalised to the mean interval. Then, linear trend removal and 10% left and right cosine tapering to zero was performed, and zeros were added to the data series until the nearest power of two. Next, the power density spectrum was computed by summation of the squared real and imaginary amplitude spectra as computed by the Fast Fourier Transformation. This power density spectrum was corrected for the losses in variance that were introduced

Table 1. Values of VO_2 max, HR_1, HR_2, HR_0, m, n, and m·n (n=44).

	Mean	SD	Min	Max
VO_2 max ($ml \cdot kg^{-1} \cdot min^{-1}$)	49.1	10.7	28.7	71.9
HR_1 (beats/min)	59.2	9.3	42.2	91.7
HR_2 (beats/min)	51.5	7.1	39.0	72.1
HR_0 (beats/min)	88.3	9.1	73.3	107.1
m	1.15	0.05	1.02	1.28
n	0.58	0.07	0.46	0.82
m·n	0.67	0.08	0.47	0.88

Fitness, heart rate before metoprolol, heart rate after metoprolol, intrinsic heart rate, sympathetic factor, vagal factor and sympathovagal balance in the studied group. VO_2 max=maximal oxygen uptake; HR_1=heart rate before metoprolol; HR_2=heart rate before atropine; HR_0=intrinsic heart rate; m=sympathetic factor; n=vagal factor; m·n=sympathovagal balance; SD=standard deviation; min=minimal value, max=maximal value.

Table 2. Values of time and frequency domain heart rate variability measures (n=44).

	Mean	SD	Min	Max
SDNN (s)	0.09	0.04	0.04	0.19
CV (%)	8.89	2.73	4.56	16.90
pNN50 (%)	37.0	20.3	20.0	73.0
RSA_{06} (s)	0.33	0.13	0.05	0.76
RSA_{15} (s)	0.13	0.07	0.02	0.32
RSA_{fr} (s)	0.14	0.07	0.02	0.37
VLF ($*10^{-3}$)	2.56	1.85	0.39	7.82
LF ($*10^{-3}$)	2.57	1.65	0.28	7.72
HF ($*10^{-3}$)	2.13	2.14	0.07	10.25
LF/HF	1.93	2.48	0.31	17.2
LF/(LF+HF)	0.58	0.14	0.24	0.94
LF/(VLF+LF+HF)	0.37	0.12	0.13	0.65

Values of the time and frequency domain HRV measures, as determined before pharmacological autonomic blockade. SDNN=standard deviation of the inter-beat-intervals between normal beats; CV=coefficient of variation; pNN50=percentage of successive inter-beat-intervals differing more than 50 ms; RSA_{06}=RSA during 6 cycles/min metronome respiration; RSA_{15}=RSA during 15 cycles/min metronome respiration; RSA_{fr}=RSA during free respiration; VLF=very-low-frequency (0.01-0.05 Hz) spectral power; LF=low-frequency (0.05-0.15 Hz) spectral power; HF=high-frequency (0.15-0.40 Hz) spectral power; SD=standard deviation; min=minimal value; max=maximal value.

Table 3. Values of rank correlation coefficients between HR_1 and all HRV measures, and m, n, or m·n (n=44).

	m	n	m·n
HR_1	0.31	0.57	0.71
SDNN	(-0.16)	-0.57	-0.66
CV	(-0.01)	-0.42	-0.47
pNN50	(-0.18)	-0.65	-0.73
RSA_{06}	(-0.05)	-0.60	-0.56
RSA_{15}	(-0.02)	-0.71	-0.73
RSA_{fr}	(-0.14)	-0.57	-0.64
VLF	(0.07)	(-0.24)	(-0.25)
LF	(-0.01)	-0.39	-0.41
HF	(-0.08)	-0.47	-0.52
LF/HF	(0.08)	(0.26)	(0.28)
LF/(LF+HF)	(0.08)	(0.26)	(0.28)
LF/(VLF+LF+HF)	(-0.13)	(0.10)	(0.06)

Values of rank correlation coefficients between HR_1 and the time and frequency domain HRV measures, and values of m, n, or m·n. Values between parenthesis are nonsignificant (significance level p=0.05). HR_1=heart rate before metoprolol, SDNN=standard deviation of the interval between normal beats, CV=coefficient of variation, pNN50=percentage of successive intervals differing more than 50 ms, RSA_{06}=RSA during 6 cycles/min metronome respiration, RSA_{15}=RSA during 15 cycles/min metronome respiration, RSA_{fr}=RSA during free respiration, VLF=very-low-frequency (0.01-0.05 Hz) spectral power, LF=low-frequency (0.05-0.15 Hz) spectral power, HF=high-frequency (0.15-0.40 Hz) spectral power.

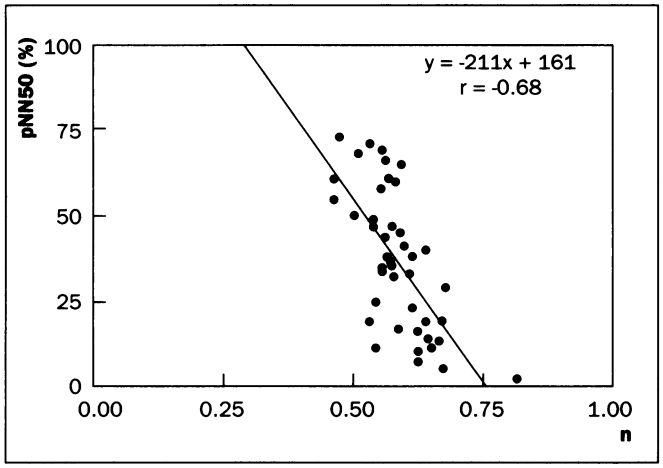

Figure 2. Scatterplot of the relation between vagal factor n and the percentage of successive intervals differing more than 50 ms (pNN50).

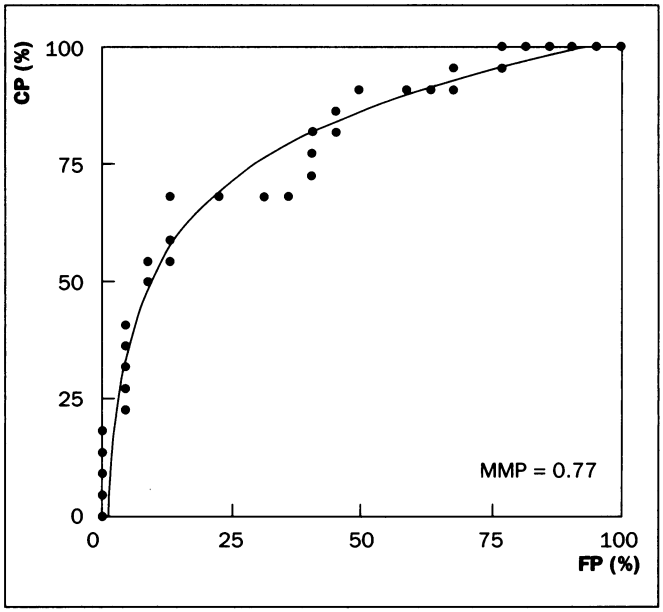

Figure 3. Receiver operating characteristic that describes the discriminative power of pNN50 in separating low and high values of the vagal factor n. MMP=maximal mean performance, FP (%)=percentage false-positive classifications, CP (%)=percentage correct-positive classifications.

by the tapering and zero padding procedures. After this correction, the very-low-frequency spectral power in the 0.01-0.05 Hz band (VLF), the low-frequency spectral power in the 0.05-0.15 Hz band (LF), and the high-frequency spectral power in the 0.15-0.40 Hz band (HF) were computed by integration of the power density spectrum over these frequency bands. Finally, the following relative spectral powers were computed: LF/HF, LF/(LF+HF), and LF/(VLF+LF+HF).

We validated HR_1 and HRV - with the pharmacologically determined sympathetic factor, vagal factor, and sympathovagal balance as gold standards - by computing rank correlations between HR_1 and all HRV measures, and m, n, and m·n. Furthermore, in an attempt to quantify the potential clinical usefulness of HR_1 and HRV as estimators of autonomic influence, we constructed receiver operating characteristics (ROCs), expressing the discriminative power of that measure in separating low and high values of m, n, or m·n. Median values of m, n, or m·n were used to arbitrarily define the low-high split point. ROCs were constructed by determining the percentages of correct-positive and false-positive classifications while increasing the discriminating value of HR_1 or the HRV measure of interest with increments of 2% of the range of that measure. As an index of merit, each point on the ROC was assigned a mean performance (= averaged sensitivity and specificity).[18] Finally, the maximal mean performance was computed for each ROC.

Results

Six subjects were excluded from the study because of vasovagal syncope shortly after venous cannulation (2 subjects), technical failure (1 subject) and episodes of nonsinus rhythm (3 subjects) during the measurement session. The remaining 44 subjects were normotensive throughout the measurement session; the spontaneous respiration rate was >12 cycles/min in all subjects. The age of the 44 subjects was (mean±SD) 25.7±3.6 years, with a height of 182.7±7.3 cm, and a weight of 75.8±8.9 kg. Six of these 44 subjects currently smoked 2, 5, 6, 15, 20, and 40 cigarettes daily, respectively.

The mean and range of VO_2 max, HR_1, HR_2, HR_0, m, n, and m·n are listed in table 1. The VO_2 max values indicate considerable differences in fitness within the studied group. In all subjects, HR_2 was lower than HR_1, and HR_0 was higher than HR_1. Time and frequency domain HRV values are listed in table 2.

The rank correlation coefficients between HR_1 and all HRV measures, and m, n, or m·n are listed in table 3. When sorted according to magnitude, LF/HF and LF/(LF+HF) have by definition, identical ranking orders. Hence, the rank correlation coefficients with m, n, and m·n are identical too (the linear correlation coefficients differ, though). It is noteworthy that HR_1 and the time domain HRV measures had larger correlation coefficients than the frequency domain HRV measures (table 3). Also, correlations with m were consistently lower than those with n and with m·n; only the correlation between HR_1 and m just reached statistical significance (significance level p=0.05). RSA during 15 cycles/min metronome breathing correlated better with n and m·n than during spontaneous or 6 cycles/min respiration. Only the absolute spectral powers LF and HF had significant, but low, correlations with n and with m·n. Separating low and high correlations at 0.71 (50% of the variance explained), only the correlations between HR_1, pNN50, RSA_{15} and m·n, and the correlation between RSA_{15} and n are noteworthy.

Table 4. Maximal mean performances of HRV measures in discriminating high and low values of m, n, or m·n (n=44).

	m	n	m·n
HR_1	0.64	0.73	0.82
SDNN	0.66	0.77	0.82
CV	0.61	0.73	0.77
pNN50	0.64	0.77	0.77
RSA_{06}	0.55	0.69	0.74
RSA_{15}	0.55	0.82	0.82
RSA_{fr}	0.64	0.75	0.80
VLF	0.57	0.64	0.64
LF	0.61	0.70	0.73
HF	0.57	0.66	0.66
LF/HF	0.59	0.52	0.55
LF/(LF+HF)	0.55	0.55	0.55
LF/(VLF+LF+HF)	0.52	0.57	0.55

Maximal mean performances (based on receiver operating characteristics) of HR_1 and HRV measures in discriminating high and low values of m, n, or m·n. HR_1=heart rate before metoprolol, SDNN=standard deviation of the interval between normal beats; CV=coefficient of variation; pNN50=percentage of successive intervals differing more than 50 ms; RSA_{06}=RSA during 6 cycles/min metronome respiration, RSA_{15}=RSA during 15 cycles/min metronome respiration, RSA_{fr}=RSA during free respiration, VLF=very-low-frequency (0.01-0.05 Hz) spectral power, LF=low-frequency (0.05-0.15 Hz) spectral power, HF=high-frequency (0.15-0.40 Hz) spectral power.

Figure 2 is a scatterplot of pNN50 versus n; figure 3 is the ROC as constructed from these data. A ROC visualises the diagnostic power (i.e. the discriminative power to separate low from high values of n) with varying threshold values. The maximal mean performance for the depicted ROC is 0.77, which is close to the best ROC values found (0.82, for HR_1, SDNN, and RSA_{15} versus m·n shown in table 4). Table 4 lists the maximal mean performances of HR_1 and of all HRV measures with respect to the discrimination of low from high values of m, n, or m·n. Overall, HR_1 and the time domain HRV measures performed better than the frequency domain HRV measures. Separating low and high maximal mean performances at 0.75 (correct classifications in 3 out of 4 cases), only HR_1 and the time domain HRV measures had diagnostic power, that is, with respect to n and m·n but not with respect to m.

Discussion

Pharmacological assessment of sympathetic and vagal tone

Gold standard

The choice of specific measures for sympathetic and vagal tone is pertinent to the validation of HR and HRV as possible noninvasive estimators of autonomic activity. For sympathetic tone - but not for vagal tone - neural firing, neurotransmitter concentrations, and neurotransmitter kinetics have been used as gold standards in humans.[10,19] For vagal tone, inter-beat-interval shortening on vagal blockade has been used as a measure.[20,21] This draws mainly on the studies by Katona et al.[22] and by Katona and Jih.[23] In the first study,[22] approximate linearity between respiration-related fluctuations in vagal firing and in inter-beat intervals was demonstrated. However, β-blockade caused 'a change in the general scale factor of the system'. In other words, the proportionality constant was dependent on the amount of adrenergic stimulation. In the second study, Katona and Jih[23] derived a theoretical linear relationship between the amplitude of respiratory sinus arrhythmia and the inter-beat-interval shortening on vagal blockade, using a linearised version of the Rosenblueth-Simeone model.[1] This relationship was then demonstrated in seven dogs. Temporary vagal blocking was realised by bilateral vagal cooling. The linear relationship for one animal was obtained by combining measurements under control conditions and under conditions that alter the autonomic state, as β-blockade or intra-aortic balloon inflation. Unfortunately, the proportionality constants differ from animal to animal, which makes these measures unsuitable for inter-individual comparisons. In other words, trends in the amplitude of the respiratory sinus arrhythmia of one individual are possibly indicative for the trends in vagal tone in this individual. But the proportionality constant is unknown, and the vagal tones of two individuals cannot be compared by comparing respiratory sinus arrhythmia or inter-beat-interval shortening on vagal blockade.

In their classical study regarding autonomic influences on sinus node automaticity from 1934, Rosenblueth and Simeone described the sympathovagal interaction by multiplying the separate sympathetic and vagal influences.[1] Since then, this model has not seriously been challenged. The alternative model as proposed by Warner and Russell in 1969[24] shows no significant discrepancies on analytical comparison.[25] The Rosenblueth-Simeone model represents the sympathetic and vagal influences by two factors, m and n, which express sympathetic acceleration and vagal deceleration of the spontaneous sinus node discharge rate. The factors m and n describe autonomic effects rather than autonomic activity. As functional measures, the Rosenblueth-Simeone factors integrate neural firing, neurotransmitter concentrations, receptor density and the properties of the intracellular signalling pathway.

Methodological considerations

The Rosenblueth-Simeone factors can be measured by pharmacological autonomic blockade. It is conceivable that variant blocking protocols (differences in drugs, doses, and blocking order) may cause differences in the thus measured values of these factors. Initial studies using pharmacological autonomic blockade

were performed with propranolol.[4,16] Later, metoprolol was also used.[3,15,26] Lewis et al.[26] report no significant differences in intrinsic heart rates when determined with propranolol or with metoprolol.

Usually, 0.2 (4×0.05) mg/kg for either propranolol or metoprolol, and 0.04 (4×0.01) mg/kg atropine are considered adequate for achieving a state of 'complete autonomic blockade' at rest. As the blocking agents compete with the endogenous neurotransmitters, such a state of complete blockade can theoretically never be reached. Practically, such a state is assumed if a subsequent bolus infusion no longer entails a change in heart rate. It is not always necessary to use four subdoses to achieve this effect. The required dosage also depends on the amount of time during which complete autonomic blockade is desired.

In our study, we used metoprolol and atropine in the maximal doses of 0.2 and 0.04 mg/kg, respectively. Metoprolol was chosen because of its cardioselectivity in an attempt to reduce side effects. Potential side effects might not only cause discomfort for the study subjects, but could also invoke unwanted effects in the unblocked - vagal - autonomic branch.

It is possible that the blocking of one autonomic branch induces compensatory activity of the opposite branch. Katona and colleagues[4] determined the values of m and n in both blocking orders. No significant differences were found, indicating that studies with sympathetic-vagal, vagal-sympathetic, or randomised blocking order would yield comparable results. In our study, we chose to block the β_1-receptors first, assuming that in the supine state vagal effects predominate. Hence, the possible decrease in vagal activity as a compensation for initial β_1-blockade may be smaller than the possible decrease in sympathetic activity as a compensation for initial blockade of the muscarinic receptors.

Intravenous cannulation is required to use the Rosenblueth-Simeone factors as gold standards for sympathetic and vagal tone means. This invasive procedure alters the autonomic state.[5] To avoid bias caused by this effect, we measured the HR and HRV control values well after the moment of cannulation (figure 1).

Autonomic characteristics of the studied group

Studies on a relation between the Rosenblueth-Simeone factors and fitness yield different conclusions. Smith et al.[3] measured a slightly lower sympathetic tone (lower value of m) and a moderately higher vagal tone (lower value of n) in athletes as compared with controls. Maciel et al.[27] found no relationship between vagal tone and aerobic capacity. Katona et al.[4] found similar sympathetic tone (equal value of m), but a diminished vagal tone (higher value of n) in athletes as compared with controls.

For our validation study, a homogeneous group with regard to the substrate, but a heterogeneous group with regard to the autonomic status would be desirable. Given the possible relation between fitness and autonomic status, we deliberately choose to include subjects of various fitness levels in the studied group. The VO_2 max values listed in m.n demonstrate that this attempt was successful. The descriptive statistics of m and n in table 1 suggest that the vagal inhomogeneity (values of n) in our group was larger than the sympathetic inhomogeneity (values of m). Correlations between VO_2 max and m, and between VO_2 max and n were low (-0.23 and -0.15, respectively), and did not reach statistical significance. It is possible that not fitness but other factors, such as differences in mental arousal due to the individual perception of the experimental conditions, may have caused the autonomic inhomogeneity in our studied group.

In our group, the intrinsic heart rate was 88.3±9.1 beats/min (table 1). This is somewhat lower than the values one might expect from the formulae by Jose and Collison:[28] based on age, a value of 103 beats/min would be expected, and based on weight this would be 99 beats/min. Other studies with groups comparable with ours as to gender, age and fitness, reported intrinsic heart rates of 83[3] and 94[4] beats/min. Hence, our intrinsic heart rate values seem to be within the range of expectation.

Respiration and heart rate variability

Respiration is a primary cause of HRV. Apart from a small direct mechanical influence,[13] breathing may induce HRV through various neural pathways. Centrally generated HRV arises from 'crosstalk' among the respiratory and vasomotor centres, and from respiratory modulation of the medullary sensitivity for baroreceptor inputs.[29-32] HRV generated by reflex mechanisms arises from respiration-induced fluctuations in the firing pattern of pulmonary stretch receptors and of baroreceptors. There is no agreement on the relative importance of each of these causative mechanisms, but several studies[33-36] tend to conclude that HRV during normal respiration is mainly of central origin.

The above-mentioned causative mechanisms of HRV all induce respiratory fluctuations in the sympathetic and vagal firing patterns. The explanation for HRV components with lower frequencies can mainly to be found in the larger time lag of, and the slower response to, the sympathetic efferent reflex response by the peripheral vasculature and the sinus node.[6,37,38] Fast respiration (>0.2 Hz) induces fast fluctuations in sympathetic firing which do not induce fast heart rate changes. Hence, the existence of a 0.1 Hz HRV component during fast respiration, and the relative increase of this component with a shifting sympathovagal balance, may be interpreted as evidence for increasing sympathetic involvement in circulatory control. With slow breathing (e.g. 0.1 Hz) both the vagal and sympathetic firing patterns and the induced

changes in heart rate will follow respiration, rendering spectral HRV analysis no longer meaningful for the distinction between sympathetic and vagal components. Monitoring respiration with spectral HRV analysis is therefore a sine qua non. In our laboratory, respiration is always continuously recorded with the ECG to verify the spontaneous breathing frequency as well as compliance with metronome breathing instructions. Spontaneous breathing frequencies appeared to be >0.2 Hz in all studied subjects.

Heart rate variability and autonomic influence

Selection of HRV parameters
The central question of the current study was how well the autonomic state (sympathetic tone, vagal tone and sympathovagal balance) can be assessed by HRV analysis. It is not obvious which HRV parameters should be selected for validation with the Rosenblueth-Simeone factors. Being generated by the same process, many HRV parameters are, to a certain extent, correlated. Indeed, significant and sometimes relevant correlations between HRV parameters have been described in the literature.[39] On the other hand, the alternative HRV parameters are not fully redundant, and contain partly independent information. Therefore, we did not restrict the number of HRV measures and studied, besides HR, the performance of six currently used[40] time and six currently used frequency domain HRV measures.

Assessment quality
The quality of the assessment of the sympathetic factor m was overall inferior to the assessment of the vagal factor n (tables 3 and 4). This finding may be caused by the autonomic heterogeneity in our group. Evidently, the inhomogeneity related to the sympathetic factor m was smaller than the inhomogeneity related to the factor n (table 1). Another explanation of the limited range sympathetic factor m is that the measurements were done in the supine recumbent position, a state associated with a relatively low sympathetic tone.

An answer to the question whether HR or any HRV measures are useful as a noninvasive estimator for the invasively determined parameters m, n, or m·n can only be given if the minimally required correlation (table 3) or maximal mean performance (table 4) is established. Obviously, statistical significance is necessary, but not sufficient. Admittedly, the threshold values as proposed in the Results section (0.71 for correlations, and 0.75 for maximal mean performances) are arbitrary choices. At the same time these threshold values represent a performance that may be sufficient for most epidemiological purposes, but that is insufficient for individual clinical diagnosis. In other words, it depends on the application whether noninvasive assessment of the autonomic status of a subject by HR or HRV may be called adequate.

Assessment of sympathetic tone
Our data demonstrate that it is quite uncertain whether sympathetic tone can be estimated from HR or HRV. The rank correlations in table 3 are disappointingly low. Only the rank correlation between the resting heart rate HR_1 and m was statistically significant, and it is unlikely that the correlation values would improve dramatically if the number of studied subjects were to be increased to also reach significance for HRV parameters. It is possible that measurements under some circulatory load (tilt, mild exercise) would yield better results. However, clinical HRV measurements are usually taken in the supine recumbent position.

Assessment of vagal tone
If vagal tone is to be estimated, RSA_{15} is the superior measure. However, given the rank correlation of -0.71 and the maximal mean performance of 0.82, it is questionable whether RSA_{15} is sufficiently reliable in assessing vagal tone.

Assessment of the sympathovagal balance
According to the Rosenblueth-Simeone equation, inter-individual differences in the intrinsic heart rate (table 1) are the only source of uncertainty when assessing the sympathovagal balance m·n from heart rate. It appeared that resting HR (HR_1), was one of the best estimators of the sympathovagal balance m·n (tables 3 and 4). However, given the rank correlation of 0.71 and the maximal mean performance of 0.82, it is questionable whether HR is sufficiently reliable in assessing the sympathovagal balance. The fact that HRV measures do not perform better than HR suggests that the physiological processes that generate HRV are at least partly different from those processes that generate the sympathovagal balance.

Time vs. frequency domain HRV parameters
The time domain HRV parameters performed better than the frequency domain HRV parameters. In a previous study, we found that within individuals the relative amount of low-frequency power measured at various tilt angles correlated very well with heart rate.[2] Obviously, there is variability between individuals as to the physiological processes that cause the low-frequency heart rate variability under conditions of high-frequency respiration.

Respiratory sinus arrhythmia
Respiratory sinus arrhythmia depends on breathing frequency and tidal volume.[41] One might then expect that RSA measures obtained during metronome respiration (RSA_{06} and RSA_{15}) would perform better than RSA_{fr} (obtained during free respiration). Due to the relatively slow response of the sinus node to fluctuations in sympathetic tone, RSA_{15} is mainly caused by fluctuations in vagal tone, while RSA_{06} may also have a sympathetic component. The fact that RSA_{15} performed best in assessing vagal tone (tables 3

and 4) is in line with this reasoning. In the light of these findings, it would be preferable to replace slow metronome respiration, which is frequently used clinically for assessment of vagal tone, by fast metronome respiration.

Limitations of the study

All measurements were done in healthy subjects. Such subjects constitute a natural group for initial validation purposes in phenomena that occur 'in health and disease'. It may be questioned what the results of a similar study would have been in patients. Disease, for example congestive heart failure[42] or coronary artery disease,[43] is generally associated with a decrease in HRV. Whether or not the Rosenblueth-Simeone factors will differ in disease is unknown; it is, for example, conceivable that elevated catecholamine levels often reported in disease are accompanied by a compensating lowered receptor density that would not result in a dramatic change in the sympathetic Rosenblueth-Simeone factor m. To resolve this issue, future research would be desirable in which a similar measurement protocol were performed in a patient cohort with evident structural heart disease.

Clinical implications

Many studies report associations of abnormal HRV values with disease or risk.[11,44,45] This at least suggests an abnormal function of the autonomic nervous system in risk-related conditions, although it is not clear whether such autonomic changes are causes or consequences of the disease. The results of our study imply that it is unjustified to strictly interpret HRV measures in terms of sympathetic tone, vagal tone, or sympathovagal balance. In this respect, the more elaborate frequency-domain HRV analysis is even inferior to the simpler time-domain analysis. Current HRV analysis techniques offer no important advantages compared with HR in assessing the sympathovagal balance, while respiratory sinus arrhythmia measured during 15 cycles/min metronome breathing yields the best assessment of vagal tone. Assessment of sympathetic tone appears not to be feasible under conditions of supine rest.

Our study demonstrates that there is definitely a relation between HRV and the Rosenblueth-Simeone factors. However, the correlations are too low to uniquely link HRV to the sympathetic or vagal tone, or to the sympathovagal balance. These findings underscore the view by Fetsch et al.[46] that 'HRV represents the integrated response of the cardiovascular system to a variety of different influences: the plasma level of catecholamines, the baroreflex activation and the direct sympathetic and vagal activity'.

These conclusions certainly do not undermine the value of HRV as a risk predictor, for example of post-infarction mortality,[47,48] or as an important factor in the genesis of life-threatening tachyarrhythmias.[49] Indeed, our study stresses the need for ongoing research towards the physiological and pathophysiological mechanisms that cause HRV. Such knowledge is especially important when risk factor modification is attempted, as in training and rehabilitation programmes.[40]

Acknowledgements

This study was supported by the Netherlands Heart Foundation (grant 43.032). The assistance of Mona Mazgani, Yvon Swier, and Janine Voogd in acquiring the data is gratefully acknowledged. ■

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