Cardiovascular Autonomic Function in Conscious Rats: A Novel Approach to Facilitate Stationary Conditions

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Background An experimental setting and software were developed to evaluate cardiovascular autonomic function in conscious rats. A restrained approach was used, which, upon proper habituation, induced little or no stress in the rats and limited motion artifacts.

Methods: The ECG and arterial blood pressure were recorded. Time- and frequency-domain indices of heart rate variability (HRV) and blood pressure variability (BPV) were calculated. The spontaneous baroreflex sensitivity (spBRS) was estimated using the method of statistical dependence.

Results: The power spectra clearly concentrated in a frequency band with center frequency around 0.4 Hz, the low frequency (LF) component, and one at the respiratory frequency at 1.5 Hz, the high frequency (HF) component. In baseline conditions, a direct association existed between mean R-R and especially HRV parameters denoting vagal modulation such as rMSSD, pNN5, and HF power. Beta-adrenergic blockade by propranolol diminished basal heart rate. Vagal indices increased while there was an exclusive decrease in the low frequency band of HRV. Alpha-adrenergic blockade with phentolamine produced a depressor response with tachycardia, and a clear decrease in the LF component of BPV. Both the LF and HF component in the HRV spectrum were virtually absent. Cholinergic blockade with atropine did not significantly alter BP but induced a clear tachycardia with decreased vagal indices. The HF component of HRV was completely abolished and the LF band was reduced.

Conclusions: Both α - and β -adrenergic blockade left spBRS virtually unaltered, while cholinergic blockade profoundly diminished spBRS. Spectral fluctuations of β -sympathetic tone were restricted to the LF range of HRV, while the HF respiratory component represented vagal modulation. The α -sympathetic system played a dominant role in the LF oscillations of BPV. A role of the vagus in the HF oscillations of BPV in the rat is questioned. The baroreflex depended mainly on changes in vagal activity.

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Rats have a widespread use in cardiovascular research. However studies of cardiovascular autonomic function in conscious rats are rather difficult,¹ mainly because of technical problems related to the registration of valid cardiovascular parameters as time series.

The conscious state is a necessary condition to study autonomic function, since all anesthetics are to some extent sympatholytic. Our initial approach included a set-up of freely moving, conscious rats by means of a tail-cuff.² The correct analysis of cardiovascular variability was frequently per-

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Figure 1. Set-up of the animal experiments in the Plexiglas restrainer (dimensions in mm)

turbed by motion artifacts, however. We therefore switched to a restrained approach and we included a rigid stationarity test in our algorithm. This method, upon habituation, induced little or no stress in the animal.³

The aims of this study were (1) to develop an experimental setting in conscious, unanesthetized rats which allows continuous ECG and BP recording and pharmacological IV and ICV interventions; (2) to establish the characteristics of HRV and BPV in time- and frequency-domain; (3) to evaluate the usefulness of power spectral analysis of HRV and BPV for the assessment of cardiac autonomic function; and (4) to assess baroreflex sensitivity (BRS) by a nonpharmacological method.

MATERIALS AND METHODS

Animal Habituation

Thirteen male Wistar rats, 3 months old and from the same origin and strain, weighing 280-300 g (obtained from the University Animal Breeding Facility, Heverlee, Belgium), were housed on a 12 to 12 light/dark cycle with dimmed lights on at 08.00 hours. Animals were allowed to eat and drink ad libitum under close monitoring of normal grooming behavior and bowel motility. For a period of 2 weeks, they were conditioned in a Plexiglas triangular restrainer (Fig. 1), before the actual experiment. Each day the rats were placed in the restrainer for half an hour. The restrainer is 20 cm

long and can be adapted for smaller rats with an end piece either at 17.5 cm or at 16 cm. The front piece has a small slit allowing the animal to breathe, and the end piece has a slit for the tail

Animal Instrumentation

At the day of the experiment, under halothane anesthesia (1% in oxygen) and with artificial ventilation (BioScience, Sheerness, Kent, U.K.; tidal volume 2 mL, respiratory frequency 75), three precordial ECG electrodes were placed subcutaneously (apex, base, right hypochonder). A self-constructed polyethylene arterial catheter (PE10 – 0.28 mm ID × 0.61 mm OD, passing on into PE40 – 0.58 mm ID × 0.965 mm OD; polyethylene tubing Intramedic, Becton Dickinson, NJ, USA) was inserted via the left femoral artery into the lower abdominal aorta for 4 cm. An IV silicone catheter with radiused tip (Intisil, 0.5 mm ID × 0.9 mm OD, Access Technologies, Ridgeway, USA) was passed via the left femoral vein into the inferior vena cava for 6 cm. Both catheters were filled with heparinized saline. After termination of anesthesia, the rats were placed in the Plexiglas restrainer in a quiet room with dimmed lights and were allowed to recover for at least 2 hours. Blood gases were checked to exclude respiratory acidosis, and values within normal range were obtained. All recordings were performed in the afternoon with a low level of illumination and with all human activity out of the animal's sight. The experiments were performed in accordance with the international directions for the protection of animals used for scientific purposes and approved by the local ethical committee.

Drug Administration

Alpha- and beta-adrenergic blockades were performed by IV administration over 1 minute of phentolamine (10 mg/kg; Regitine, Novartis Pharma, Basel, Switzerland) or propranolol (4 mg/kg; Inderal, Zeneca, Wayne, NJ, USA), respectively. Efficacy of the dose of blockades was assessed by the measurement of the pressor response to an IV bolus injection of the α -adrenergic agonist phenylephrine (2 μ g/kg, pharmacy University Hospital Leuven, Belgium) and the heart rate response to an IV bolus injection of the β -adrenergic agonist isoprenaline (1 μ g/kg, Isuprel, Abbott, Chicago, IL, USA). Cholinergic blockade was performed by the

IV administration of atropine sulfate (1 mg/kg, Ste-rop, Brussels, Belgium).

Recordings were made up to 30 minutes after IV administration of the antagonist(s).

Of the group of 13 rats, 4 received α -adrenergic blockade, 5 β -adrenergic blockade, and 4 cholinergic blockade without any crossovers.

Recording durations (baseline and after pharmacological interventions) were obtained between 10 and 20 minutes.

Data Acquisition

The arterial catheter was connected to a Baxter Uniflow pressure transducer (Baxter, Brussels, Belgium) and the blood pressure (BP) signal amplified by a Siemens pressure amplifier 863 module. The ECG signal was amplified on a Siemens Mingo-graph 82. Analogue/digital conversion was done with an external A/D convertor (Dataq DI 220PGH, 8 channels, 12-bit precision, maximal 82.9 kHz sampling rate over all channels, DATAQ Instruments Inc., Akron, Ohio, USA). A PC, equipped with Windaq recording software and advanced Codas (DATAQ Instruments Inc.), performed real-time digital signal acquisition to hard disk and to a read/write CD-ROM. The sampling rate was 1000 Hz per channel, thus giving a time resolution for the R-R intervals of 1 ms.

Data Analysis

Time-Domain

Importation and peak detection of the Windaq files with the ECG and BP signals was performed with the program ACTS⁴ (Automated Calculation of Tachograms and Systograms), that was written in LabVIEW 4.0.1. HRV and BPV parameters were calculated in general agreement with the standards of measurement recently proposed by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology.⁵ The discrete time series values of R-R or SBP intervals (systolic blood pressure) derived from ACTS were used as input to the LabVIEW program Main Analysis 2.0. Premature ectopic beats or artifacts produce short beat-to-beat intervals, sometimes followed by a compensatory delay. Both these disturbances induce a sharp transient peak in the tachogram and can lead to major errors when analyzing HRV data in both time- and frequency-domain. These errors were eliminated by careful editing and visual inspection of the signals.

In case of ectopic beats, two linear filters could be applied to correct for data points outside a limit interval: a percentage filter (default at 20%) replaced every value that deflected more than 20% of the previous one by the mean value of the five preceding and the five following values. Another filter could be applied that controlled values with a larger deflection than 3 standard deviations (3 SD) from the mean value. This aberrant value was again replaced by the mean value of the five preceding and the five following beats. The assumption underlying this correction is that the R-R interval does not oscillate abruptly from the mean value and that the use of linear interpolation will introduced less high frequency noise.

Parameters in the time-domain were determined on the original or filtered tachogram: mean and standard deviation (SD) of R-R tachogram, maximum and minimum of R-R values, the square root of the mean of sum of the squares of differences between adjacent R-R intervals (rMSSD), percentage of intervals that vary more than 10 or 5 ms from the previous beat (pNN10, pNN5), (value of 5 ms was selected due to the high mean heart rate in rats: 300 to 500 beats/min), and the standard deviation of the 1-minute average of R-R intervals (SDANN1). Finally a histogram was depicted (Fig. 2).

Frequency-Domain

A cubic spline approximation⁶ was used for re-sampling at 0.1 s intervals. This method allowed working with an exact frequency scale expressed in Hz. The scaled amplitude spectra of the original and resampled curves are identical. The original tachograms were used to determine all time-related and statistical parameters and the resampled ones only for computation of the power spectra.

During each intervention, recordings were made during 10 to 20 minutes, and data sets consisting of 256 points (this corresponds to a time window of 25.6 s), overlapping by 50% were processed.⁷ This method is based on multiple computations and averaging of the fast Fourier transform (FFT) of overlapping data segments. With this method, the variance of the estimated power spectral density is reduced by a factor proportional to the number of data segments used (approximately 47). For each data set, the DC component was removed from the data segment, by subtracting the mean value and a Hanning window was applied. No filtering was employed. Before performing Fourier analysis on

Figure 2. Illustration of the Main-analysis algorithm and different steps in the analysis of HRV, starting from ECG (upper left panel) and RR interval point series to R-R time series (right panel). The time-domain analysis is on the right and the frequency domain analysis on the resampled signal on the right lower panel. A histogram is depicted on the lower left panel.

finite-length data, windows have to be used to minimize the transition edges of the truncated waveforms, thus reducing spectral leakage. Power spectral density (in ms^2/Hz or mmHg^2/Hz) was then computed using a fast Fourier transform (FFT) algorithm. The frequency resolution⁸ was $\Delta f = 1/(N \cdot \Delta t)$ or 0.039 Hz (N ; number of points (256); $\Delta t = 0.1$ s, time resolution). The maximum frequency was $f_{\text{max}} = \Delta f \cdot N/2$ or 5 Hz.

Two frequency bands were defined, adapted to the heart rate of rats:^{9,10,11,12,13} a low frequency (LF) band from 0.195 to 0.74 Hz and a high frequency (HF) band from 0.78 to 2.5 Hz. Within each frequency band power was computed by integration of the power spectral density function, in absolute values of low, high, and total power (in ms^2 or mmHg^2), and a low-to-high ratio. With FFT methods it is often impossible to identify isolated

spikes, therefore no efforts were made to identify separate spikes.

To check the stationarity of the time series two different methods were used: (1) a parametric test: the mean value and the standard deviation (SD) of the total recording as well as of each data segment of 256 points were calculated. It was controlled that the variation of the mean value from one segment to another was less than one SD, and the method has been implemented in our analysis algorithm; (2) a nonparametric test: a "run" test.¹⁴ In this case a criterion has to be met. Both procedures have been explained extensively in previous work.

Baroreflex Sensitivity

The spontaneous baroreflex sensitivity was estimated with the method of statistical dependence

between SBP and HR values that relies on the estimation of probabilities.¹⁵ Briefly, the Z coefficient was computed on (SBP, HR) couples made of HR and SBP values observed on the same cardiac beat. The width of the intervals used for SBP were 3 mmHg and for heart rate 2 beats/min. Data were then divided in 15 intervals between maximum and minimum values and probabilities were estimated with the observation frequencies of at least 10. The couple with the highest probability, called modal class, was taken as a set point of autonomic cardiovascular activity. The results of the Z analysis were depicted in a matrix containing for each (SBP, HR) couple the number of cardiac beats with such values and the estimated Z value. Four quadrants were defined from the modal class taken as the origin of two modal axes. Significant couples ($Z(\text{SBP, HR}) > 0$) in the two quadrants including low SBP with high HR values (quadrant 1) and high SBP associated with low HR values (quadrant 3) were shown to be related to baroreflex activity, and couples with high SBP and high HR (quadrant 4) to sympathetic activity. Baroreflex sensitivity could be approximated by a linear regression of the weighed (SBP, HR) couples.¹⁶ To estimate contributions in specifically the low frequency oscillations, the calculations could be performed after low-pass filtering (cut-off frequency 0.75 Hz) of SBP and HR time series with a fifth-order Butterworth filter.

Statistical Analysis

Statistical analysis was performed with SPSS for Windows Release 7.5 (Scientific Packages for Social Sciences, Inc., Chicago, Illinois, USA). The Shapiro-Wilk test was used to test normality. Because of their skewed distribution rMSSD, total power, LF power, HF power, and LF/HF ratio were transformed by calculating their natural logarithm. For pNN5 and pNN10, we used the square root as transformation. Differences within groups, before and after IV pharmacological blockade, were analyzed by paired *t*-tests. Differences between groups were analyzed by multivariate analysis of variance (MANOVA) with Bonferroni correction for multiple measurements. To test the association between the different indices in baseline conditions, a two-tailed Pearson correlation coefficient (*r*) was calculated. Correlations (used in comparing parameters) from 0 to 0.25 (or -0.25) were disregarded since, in biomedical sciences, they indicate only little or no

relationship.¹⁷ Data are expressed as mean $\pm$ SE, unless mentioned otherwise. Differences with a $P < 0.05$ were considered statistically significant.

RESULTS

The Main-Analysis algorithm is illustrated in Figure 2 with the tachogram and its resampled version and calculated HRV parameters from a representative animal. Individual and mean ($\pm$ SD) time- and frequency-domain data for all rats in basal conditions are summarized in Table 1. The mean R-R interval of 165 ms corresponds to a mean heart rate of 364 beats/min.

Continuous recordings of ECG and BP in conscious, restrained rats gave power spectra of HR and BP fluctuations, clearly concentrated in two frequency bands: one with center frequency around 0.4 Hz, the LF component, and one at the respiratory frequency at about 1.5 Hz, named HF component (Figs. 3 and 4).

In baseline conditions, a direct association between mean R-R and especially HRV parameters denoting vagal modulation, such as rMSSD, pNN5 and HF power, existed (Table 2, first column). No correlation with SD, SDANN1, or LF power was present. As expected, the different time-domain vagal indices pNN5 and rMSSD correlated very well ($r = 0.97$), as did the global variability reflecting time-domain parameters SD and SDANN1 ($r = 0.97$). rMSSD and pNN5 correlated with the frequency-domain HF power ($r = 0.98$ and 0.95 , respectively). The frequency-domain parameter total power showed a strong association with LF power ($r = 0.95$) and with HF power ($r = 0.82$). LF power was only moderately associated with HF power ($r = 0.66$). Mean SBP only correlated with rMSSD ($r = -0.62$, $P < 0.05$). SD of BPV was related to total and LF power, and to LF/HF ($r = 0.87$, 0.78 , and 0.77 , respectively, $P < 0.01$). rMSSD correlated with pNN5 ($r = 0.82$, $P < 0.01$), but both indices were not related to HF power in the frequency-domain. Total power was nearly exclusively determined by LF power and not HF power ($r = 0.97$, $P < 0.01$, versus nonsignificant). Quite intriguing, lower mean R-R (higher heart rate) was associated with a higher total and LF power in BPV ($r = -0.92$ and -0.94 , respectively, $P < 0.01$), and lower high frequency HRV (rMSSD, pNN5, and HF power) was associated with (exclusively) higher LF power in BPV ($r = -0.60$, -0.66 , and -0.61 , respectively, $P < 0.05$).

Table 1. Normal Values of Autonomic Indices of HRV, BPV, and Spontaneous BRS in Baseline Conditions in 13 Conscious Rats

	Mean	Standard Deviation	25 th Percentile	75 th Percentile
HRV				
Mean RR (ms)	164.70	24.59	145.35	173.32
SD (ms)	8.36	4.57	5.64	10.31
SDANN1 (ms)	5.49	4.33	3.04	5.91
rmSSD (ms)	4.41	2.38	2.65	5.09
pNN5 (%)	15.85	16.26	3.58	21.59
Tot power (ms ²)	20.28	2.41	11.13	41.67
LF power (ms ²)	5.21	2.69	2.80	13.46
HF power (ms ²)	3.63	3.15	1.47	6.75
LF/HF	1.98	1.42	0.80	3.47
BPV				
Mean SBP (mmHg)	126.52	7.67	120.36	132.64
SD (mmHg)	4.36	0.73	3.66	4.90
SDANN1 (mmHg)	1.81	0.60	1.28	2.31
rMSSD (mmHg)	2.26	0.25	2.02	2.49
pNN5 (%)	2.42	1.47	1.11	4.03
Tot power (mmHg ²)	13.32	1.63	10.17	19.10
LF power (mmHg ²)	8.49	1.87	6.75	13.73
HF power (mmHg ²)	1.19	1.49	—	1.53
LF/HF	8.68	5.05	4.86	12.71
BRS				
Slope (beats/min/mmHg)	-2.76	1.18	-2.09	-3.24

Figure 3. Representative example of the effect of β -adrenergic blockade on HRV (upper panel) and BPV (lower panel) spectral analysis (left: baseline conditions, right: propranolol).

Figure 4. Representative example of the effect of α -adrenergic blockade on HRV (upper panel) and BPV (lower panel) spectral analysis (left: baseline conditions, right: phentolamine).

Figure 3 shows the effects of β -adrenergic blockade by propranolol on HRV spectral analysis. The basal heart rate diminished from 363 beats/min to 297 beats/min. In time-domain rMSSD (5.52 ± 1.16 ms and 2.62 ± 0.12 mmHg) and pNN5 ($27.67 \pm 1.77\%$ and $3.95 \pm 0.55\%$) for HR and for SBP, respectively, significantly increased ($P < 0.05$), compared to baseline conditions. The total energy was reduced on the HR power spectrum and there was a significant decrease exclusively in the low frequency band of HRV (Fig. 5), leaving the LF

(and HF) component in SBP spectral analysis virtually unaffected (Table 3). Alpha-adrenergic blockade with phentolamine produced a depressor response (76 mmHg) with accompanying tachycardia (472 beats/min) and a clear decrease in the LF component of BPV (Fig. 4). The HRV spectrum presented with a significant reduction in total energy, rendering both the LF and HF component virtually absent (Fig. 6). The effects of cholinergic blockade by atropine are shown in Figure 5. Cholinergic blockade did not significantly alter BP but

Table 2. Correlations between the Different HRV Time and Frequency Domain Indices

r	Mean RR	SD	SDANN1	rMSSD	pNN5	Tot Power	LF Power	HF Power
SD	NS							
SDANN1	NS	.97**						
rMSSD	.62*	NS	NS					
pNN5	.65*	NS	NS	.97**				
Tot power	.58*	.76**	.60*	.80**	.75**			
LF power	NS	.82**	.71**	.62*	NS	.95**		
HF power	.63*	.56*	NS	.98**	.95**	.82**	.66*	
LF/HF	NS	NS	NS	NS	NS	NS	NS	NS

* Correlation is significant at the 0.05 level (2-tailed); ** correlation is significant at the 0.01 level (2-tailed).

Figure 5. Representative example of the effect of cholinergic blockade on HRV (upper panel) and BPV (lower panel) spectral analysis (left: baseline conditions, right: atropine).

induced a clear tachycardia (434 beats/min) with decrease of rMSSD and pNN5. Atropine markedly reduced total spectral energy of HRV. The high frequency component of HRV was completely abolished and the low frequency band was markedly reduced (Fig. 6, Table 3). In the BP spectral analysis, the LF and HF component possibly tended to be slightly reduced, but both remained clearly recognizable (Fig. 5).

Average values of baroreflex sensitivity are depicted in Table 1. Propranolol tended to lower but did not significantly affect the spBRS, and phentolamine had no influence on spBRS. Atropine drastically blunted the BRS slope (Fig. 6, Table 3). Application of the low-pass Butterworth filter did not significantly alter the BRS slope (-2.61 ± 0.42 beats/min/mmHg) and the same alterations were seen after the different pharmacological blockades.

DISCUSSION

This study suggests that the proposed setup consisting of a conscious rat placed in a restrainer, is a valid model to study autonomic cardiac function. Besides restraint, also swivel and telemetry set-ups exist to obtain ECG and blood pressure. These have recently been compared for blood pressure measurements.¹⁸ Telemetry methods still have some drawbacks: rather difficult implantation

in small animals and drift in telemeter offset and need for recalibration for blood pressure. With our method we obtained similar results for mean heart rate and pressure in the control situation as obtained with a swivel, and our own previous results,^{9,13,16} as well as from telemetry studies.^{10,19}

Frequency Spectra

Like in different individuals in all species,²⁰⁻²² resting heart rate varies. On the one hand, the mean R-R duration as obtained in this study (165 ms) is within the range as given by several authors in unrestrained conscious rats: 175 ms,²⁰ 171 ms,¹³ 194 ms,¹⁰ 158 ms,¹ 140-188 ms,⁹ including our own previous results (174 ms).² Spectral data, on the other hand, show a larger dispersion or even give some contradictory results. This is probably due to the use of other methodologies (such as autoregressive modeling with peak identification) but most by the choice of different frequency bands with limits from 0.195 to 0.6 Hz (LF band) to 0.6 to 2.5 Hz (HF band) and a ratio between low and high frequency bands of 0.32,¹³ and from 0.02 to 0.195 Hz (LF band) to 0.195 - 0.6 Hz (HF band) and a ratio of 3:5.⁹

The same two major regions of power are observed in both HRV and BPV power spectra of restrained conscious rats: one at the lower end of

Table 3. Effect of Pharmacological Blockades on Heart Rate, Blood Pressure and their Spectral Analysis Components

Treatment	Mean R-R (ms)	LF Power HRV (ms ²)	HF Power HRV (ms ²)	LF/HF HRV	Systolic BP (mmHg)	LF Power BPV (mmHg ²)	HF Power BPV (mmHg ²)	LF/HF BPV	SpBRS (bpm/mmHg)
Control (n = 13)	165 ± 7	5.2 ± 1.3	3.6 ± 1.4	1.98 ± 0.39	126 ± 2	8.5 ± 1.2	1.2 ± 1.1	8.68 ± 1.40	-2.76 ± 0.32
Propranolol 4 mg/kg (n = 5)	202 ± 19*	2.4 ± 1.6*	6.3 ± 1.3	0.42 ± 0.09**	131 ± 4	5.9 ± 1.6	1.3 ± 1.1	6.92 ± 1.95	-1.51 ± 0.28
Phentolamine 10 mg/kg (n = 4)	127 ± 3*	0.05 ± 1.91*	0.35 ± 1.53	0.65 ± 0.58*	76 ± 9*	0.63 ± 1.35*	2.2 ± 1.5	0.31 ± 0.07*	-2.15 ± 0.19
Atropine 1 mg/kg (n = 4)	138 ± 2*	0.33 ± 0.13**	0.78 ± 1.54*	0.24 ± 0.10*	123 ± 3	4.5 ± 1.5	1.1 ± 1.1	4.50 ± 1.13	-1.03 ± 0.06*

Paired statistics baseline vs. pharmacological blockade * P < 0.05, ** P < 0.01.

the spectrum, centered around 0.4 Hz, and in accordance with others,^{9,23} (but not with Akselrod et al.²¹) who found it between 0.10 and 0.30 Hz in Wistar-Kyoto rats); the other at the respiratory peak, usually between 1-1.5 Hz, in agreement with previous findings in Wistar rats.⁹ These frequencies are consistent with the expected higher metabolic rate of the rat, with faster physiological control loops than in larger species, e.g., compared with 0.1 Hz (LF) and 0.3 Hz (HF) in humans.²² The fast HR and relatively high power in the frequency range < 1.0 Hz suggest a high level of sympathetic control in the rat. On the other hand, mean R-R seems to be determined predominantly by vagal modulation since only correlations with vagal indices were found. Synchronization of breathing and heart rate (increase of heart rate during inspiration and decrease during expiration) has since long been a landmark^{23,24} and has often been used to establish physical fitness.²⁵ The HF frequency peak depends on respiratory rate since in the anaesthetized and artificially ventilated rat, we found its frequency at 1.25 Hz to match exactly the respiratory rate of 75 breaths per minute, giving also an absolute calibration of the frequency axis.

Pharmacologic Interventions

Spectral fluctuations of β -sympathetic tone are restricted to the low frequency range of the HRV spectrum while the respiratory peak remained mostly unchanged after β -adrenergic blockade. This observation suggests that respiratory sinus arrhythmia is purely vagal in rats. Indeed, β blockade may have a minor effect on LF of HRV in conscious dogs,²⁴ but might have a more marked effect in conscious rats,^{9,26} indicating species differences. Different effects have also been reported between chronic and acute β -blockade.²⁷

Furthermore, atropine was responsible for a dramatic decrease in the power spectrum at high as well as at low frequencies. The high frequency respiratory component completely disappeared, which proves that it represents vagal modulation. The simultaneous reduction of low frequency power indeed shows that the spectral content of the LF component(s) is far more complex than is often maintained. Next to the contribution of β -adrenergic activity to the LF band, the exact involvement of other neurotransmitter systems, such as the cholinergic and the renin-angiotensin-aldosterone system, remains unresolved.

Figure 6. Boxplots of the LF and HF power for BPV (upper panel) and HRV (middle panel) showing the effect of β -adrenergic blockade (propranolol), α -adrenergic blockade (phentolamine), combined α - and β -adrenergic blockade, and cholinergic blockade (atropine sulfate) and baroreflex sensitivity (lower panel).

The LF component of BPV, which has been attributed to fluctuations of vasomotor origin,²⁸ is reduced significantly after α -adrenergic blockade, indicating that the α -sympathetic system plays a dominant role in the LF oscillations. In addition, however, phentolamine also drastically reduced the LF component of HRV. The BPV remained virtually unaltered after cholinergic blockade, suggesting a lack of a direct influence of the parasympathetic system.²³ Others¹² have reported that chronic (chemical) sympathectomy also drastically decreased LF power in BPV and HRV. The HF component of BPV is a matter of concern, in so far that the fluctuations in vascular resistance, mainly mediated solely through the sympathetic nervous system, are incapable to reach frequencies as high as 1.5 Hz. To rationalize this finding, we recall that BP fluctuations can be secondary or coupled to HR fluctuations or may arise independently from HR

as a result of fluctuations in peripheral resistance (this holds true for the LF band).²⁹ The HF component of HRV is almost completely abolished by atropine, demonstrating the respiration-dependent control by the parasympathetic system. The HF component of BPV however, is unaffected by atropine, questioning a buffering role of the vagus in these oscillations in the rat. In our approach, no distinction was made between the so-called low and mid-frequency range in the LF power band. One evident reason is the virtual absence of a mid-frequency component in the majority of the HR and BP power spectra in our results. It cannot however, be excluded that a certain physiological role may be attributed to this mid-frequency component of HRV in the future since it shows a clear elicitation and/or increase after IV administration of an angiotensin converting enzyme-inhibitor (data not shown).

Baroreflex Sensitivity

In the last years increasing attention has been paid to the quantification of feedback mechanisms such as BRS, secondary to the emerging clinical applications.³⁰ We successfully implemented this method of statistical dependence to quantify spBRS, which correlated very well with the phenylephrine and nitroglycerine-method in the rat.¹⁶ A major advantage of this method was the absence of any pharmacological interference, which circumvents possible side effects, and of the need for an extra IV access. It has been clearly shown that pharmacological agents such as phenylephrine are no useful agents to assess baroreflex function in the rat due to its marked cardiac stimulant actions.³¹ Furthermore, phenylephrine induces changes in venous compliance and venous return and may stimulate baroreceptor pathways regardless of the increase in arterial pressure.³² In other circumstances, such as after the administration of phen-tolamine, a clear tachycardia is already present, questioning the applicability of the phenylephrine method. Our results showed that spBRS after phen-tolamine still showed valid results.

Our results on autonomic indices of restrained conscious rats extend the few previous reports including our own results on HRV in unrestrained conscious rats, and no major differences could be encountered. The restrained approach with habituated animals certainly offers advantages on the level of stationarity, since the application of spectral analysis and BRS may otherwise be precluded due to motion artifacts. This confirms the validity of our set-up, which is easy to use and very cheap compared to other methods and devoid of motion artifacts, but requires a (pivotal) habituation of the rats to restraintment.

Limitations of the Study

1. A possible stress effect due to the use of a restrainer cannot be totally excluded. However, this influence should be minimal due to the fact that comparable heart rate values were found compared to other methods.
2. For ethical reasons and out of respect for living animals as well as for stringent rules for the use of laboratory animals, only a limited number of rats could be used for this study.
3. Theoretically telemetered animals are to be preferred as a model for free moving animals. How-

ever for these experiments this was not possible as a continuous drug infusion was required.

CONCLUSIONS

In summary, our values of spBRS in rats extended the results obtained in an initial report describing the technique¹⁶ and we showed that both α - and β -adrenergic blockade left BRS virtually unaltered, while cholinergic blockade profoundly diminished BRS, indicating that the baroreflex cardiac responses (measured by spBRS) depended mainly on changes in vagal activity, and did not express baroreflex vascular responses mediated by sympathetic activation. The possibility of analyzing BRS under spontaneous conditions remains intriguing and offers clear advantages, such as the fact that the test can be repeated several times and under different experimental conditions.

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