NON-LINEAR ANALYSIS OF HEART RATE AND BLOOD PRESSURE VARIABILITY AFTER AUTONOMIC BLOCKADE IN RATS

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Abstract: This study assesses the effects of autonomic blockade (α- and β-adrenergic and cholinergic) on cardiovascular function using heart rate variability (HRV), blood pressure variability (BPV) and baroreflex sensitivity in rats using non-linear dynamics.
In 13 rats, heart rate and aortic blood pressure were measured continuously before, during and after autonomic blockade with atropine, phentolamine and propranolol. Non-linear scaling properties were studied using: 1/f slope, fractal dimension, and long- and short-term correlation. Non-linear complexity was described with correlation dimension, Lyapunov exponent and approximate entropy. Non-linear indices were compared to linear time and frequency domain indices.
β-blockade did not alter the non-linear characteristics of HRV and BPV. Low frequency power of HRV was depressed. α-blockade decreased scaling behaviour of HRV, while cholinergic blockade decreased the complexity of non-linear HRV. For BPV the scaling behaviour was increased during α-blockade and the complexity was increased during cholinergic blockade. Linear indices of HRV and BPV decreased.
Our results indicate that the β-adrenergic system has little involvement in generating non-linear HRV and BPV in rats. Cholinergic blockade decreases complexity measures in HRV. α-blockade influenced the scaling properties of the time series, while cholinergic blockade induced changes in the complexity measures.

Introduction

Since the initial publication by Akselrod et al in 1981[1], describing the relation between spectral components of heart rate variability (HRV) and sympathetic and vagal modulation, a vast amount of studies has emerged describing the influence of the autonomic nervous system on HRV[2,3]. A different approach to detect regularity or irregularity of cardiovascular function is through analysis of non-linear dynamics [4-6].

The complexity of a system can be estimated by calculating its behaviour in phase space. The dimension of the phase space (correlation dimension[7,8]), the sensitivity to initial conditions (Lyapunov exponent[9]) and the system entropy (Approximate Entropy[10,11]) are some of the indices that describe non-linear complexity behaviour.

Another non-linear property of a system is scaling behaviour. It has been shown that normal spectra of HRV exhibit a $1/f^{\beta}$ behaviour[12] ($-\beta$ = slope). This 1/f behaviour is a property of scaling or fractal behaviour and is affected under various pathological conditions[13] and by ageing[14]. A fractal has the essential characteristic that its details at a higher scale are similar (though not necessarily identical) to those of the structure seen at larger or smaller scales. Detrended fluctuation analysis quantifies fractal-like correlation properties of the time series[15]. The short- and long-term scaling exponents (or self-similarity parameters) can be obtained using the method of detrended fluctuation analysis. The spatial occupancy of the time series can be quantified using the fractal dimension[16].

Especially with the increased use of non-linear dynamics in clinical studies, it is important to understand the physiological mechanisms underlying the generation of these fluctuations.

The aim of the present study was: 1) to examine the influence of several autonomic blocking agents on non-linear HRV and BPV indices, and 2) to explore the physiological mechanisms contributing to these non-linear fluctuations, by relating them to well-known time and frequency domain indices and the spontaneous baroreflex index (spBRS).

The influence of different pharmacological interventions was studied in rats on non-linear complexity measures (correlation dimension (CD), the largest Lyapunov exponent (LE) and approximate entropy (ApEn)) and non-linear scaling indices (fractal dimension (FD), 1/f-slope, and long- (DFAα2) and short-term (DFAα1) fluctuations using the detrended fluctuation algorithm). These non-linear indices were compared with standard time and frequency domain HRV and BPV parameters.

Materials and Methods

Animal preparation: Thirteen male Wistar rats weighing 280-300 g (obtained from the University Animal Breeding Facility, Heverlee, Belgium) were conditioned in a Plexiglas triangular restrainer for at least two weeks before the actual experiment. Each day the rats were placed in the restrainer for at least one

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hour. This technique has been described in more detail in a previous publication[22]. On the day of the experiment, 3 precordial ECG-electrodes were inserted subcutaneously (apex, base, right hypochonder) under short halothane anaesthesia (1% in oxygen) and with artificial ventilation (BioScience, Sheerness, Kent, U.K.; tidal volume 2 ml, respiratory frequency of 75/min). A poly-ethylene arterial catheter (PE10–0.28 mm ID x 0.61 mm OD, introduced into PE40–0.58 mm ID x 0.965 mm OD; poly-ethylene tubing Intramedic, Becton Dickinson, NJ, USA) was inserted via the left femoral artery into the lower abdominal aorta for 4 cm. An i.v. silicone catheter with radiused tip (Intisil, 0.5 mm ID x 0.9 mm OD, Access technologies, Ridgeway, USA) was introduced via the left femoral vein into the inferior vena cava for 6 cm. Both catheters were filled with heparinised saline. After termination of anaesthesia, the rats were placed in the Plexiglas restrainer in a quiet room with dimmed lights and were allowed to recover for several hours (minimum 2). This was sufficiently long to exclude the effects of the anaesthesia, since the recovery after halothane anaesthesia has been reported to be about 10 minutes in rats[23,24]. After checking blood gasses to exclude respiratory acidosis and obtaining values within normal range, the recordings were performed. A low level of illumination was maintained and all human activity was performed 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.

α- and β-adrenergic blockade were performed by i.v. administration over 1 min of phentolamine (10 mg/kg; Regitine, Novartis Pharma) or propranolol (4 mg/kg; Inderal, Zeneca), respectively. Efficacy of the dose of blockades was assessed by the measurement of the pressor response to an i.v. bolus injection of the α-adrenergic agonist phenylephrine (2 μg/kg, university hospital pharmacy) and the heart rate response to an i.v. bolus injection of the β-adrenergic agonist isoprenaline (1 μg/kg, Isuprel, Abbott). Cholinergic blockade was performed by the i.v. administration of atropine sulphate (1 mg/kg, Sterop). Recordings were made up to 30 min. after i.v. 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 crossover.

Data acquisition: The ECG signal was amplified on a Siemens Mingograph 82. 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. Analogue/digital conversion was performed with an external Dataq A/D convertor (Dataq DI 220PGH, 8 channels, 12 bit precision, maximal 82.9 kHz sampling rate over all channels, DATAQ Instruments Inc., Akron, OH, USA). Recordings of at least 20 minutes after autonomic blockade were sufficiently long to calculate non-linear indices because of the high heart rate in rats. Each recording contained at least 7000 RR-intervals. The sampling rate was 1000 Hz per channel. RR-intervals and systolic blood pressure values were calculated[17] and after visual inspection exported for further analysis.

Data analysis: The non-linear characteristics of HRV and BPV were computed using different methods. These can be divided into 2 categories: indices that describe the scaling behaviour of the non-linear system (FD, 1/f, DFAα1 and DFAα2) and indices that describe the complexity of the system (CD, LE, ApEn).

Linear HRV and BPV parameters were calculated in agreement with the standards of measurement proposed by the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology[18]. After resampling of the tachogram/systogram at 10 Hz using a cubic spline approximation (for details see Aubert et al[19]) power spectra were obtained using fast Fourier transformation. The DC component was removed by subtracting the mean value of the data set. Recordings were made for at least 20 minutes after the intervention and a Hanning window of 256 points (corresponding to 25.6 s, gliding window with 50% overlap) was used. Two frequency bands were defined, taking into account the higher heart rate of rats[20-24]: 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 the spectral power was expressed in absolute values of low, high, and total power (in ms^2 for HRV and in $mmHg^2$ for BPV), and a low-to-high ratio. LF and HF power in normalised units (n.u.) were also calculated (LFn.u. = LF/(LF+HF) and HFn.u. = HF/(LF+HF)).

The spontaneous baroreflex sensitivity was estimated with the method of statistical dependence between systolic blood pressure (SBP) and HR values that relies on the estimation of probabilities[25]. The method allows calculating a coefficient of dependence. This coefficient is based on the number of occurrences of a couple (SBP, HR). Couples with low SBP values and high HR and couples with high SBP associated with low HR values were shown to be related to baroreflex activity. Baroreflex sensitivity can be approximated by a linear regression of the weighted (SBP, HR) couples. No time lag was used between HR and SBP values to create the couples. This method has several advantages over pharmacological interventions. No i.v. drug administration is necessary and BRS can be measured in the normal physiological range over a long period of time. It presents a steady state measurement of the baroreflex under stationary conditions.

Statistics: Statistical analysis was performed with SPSS for Windows version 8.0 (Scientific Packages for Social Sciences, Inc., Chicago, IL, USA). Normality of distribution was tested using the Shapiro-Wilk test. Because of their skewed distribution pNN5, rMSSD, total power, LF power, HF power and LF/HF ratio were transformed by calculating their natural logarithm. Differences within groups, before and after i.v. pharmacological blockade, were analysed by paired t-

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tests. Differences between groups were analysed by multivariate analysis of variance (MANOVA) with Bonferoni correction for multiple measurements. Data are expressed as mean+SD. Differences with $p<0.05$ were considered statistically significant.

Results

Table 1. Overview of the behaviour of non-linear indices after blockade.

	β-blockade	α-blockade	Cholinergic blockade
		HRV	
Scaling behaviour	=	↓	=
Complexity behaviour	=	=	↓
Low Frequency power	↓	↓	↓
High Frequency power	=	=	↓
		BPV	
Scaling behaviour	=	↑	=
Complexity behaviour	=	=	↑
Low Frequency power	=	↓	=
High Frequency power	=	=	=

↓: decrease compared to baseline

↑: increase compared to baseline

=: no difference compared to baseline

β-blockade by propranolol: An increase in mean RR interval was observed from 166 ms to 202 ms (corresponding to a decrease in heart rate from 361 bpm to 297 bpm).

Total spectral energy decreased after β-blockade ($p<0.05$), mostly caused by a reduction in LF spectral power ($p<0.05$). This was reflected in the decrease in LFn.u. and the LF/HF ratio. Mean SBP did not change significantly. No changes were observed in the linear BPV indices. Spontaneous baroreflex sensitivity was not altered.

The non-linear indices of HRV remained unchanged, except an increase in ApEn was observed ($p<0.05$). In BPV the 1/f slope was steeper and the DFAα2 index increased (both $p<0.05$).

α-blockade by phentolamine: α-blockade produced a significant tachycardia (heart rate from 375 bpm to 472 bpm; $p<0.05$). A significant decrease in linear HRV occurred, again for the LF power of HRV, although the change in LFn.u. did not reach statistical significance. Also for BPV a significant reduction in LF power was noted, while mean SBP significantly decreased. LFn.u. decreased significantly ($p<0.001$). SpBRS remained unchanged.

Non-linear scaling HRV indices decreased (DFAα1, DFAα2 and FD). The ApEn value again increased. In BPV, both DFA indices were closer to 1, indicating a higher degree of non-linear behaviour (DFAα1 $p<0.001$; DFAα2 $p<0.05$). The proportion of files in which the CD of the surrogate data sets was different from the CD of the original data also increased to 50%. No significant differences were observed in S-value and the Lyapunov exponent.

Cholinergic blockade by atropine: Cholinergic blockade induced a tachycardia (heart rate from 355 bpm to 437 bpm; p=NS) with a decrease in pNN5 ($p<0.05$). The total spectral power of HRV was reduced, represented in a decrease in both LF and HF ($p<0.01$ and $p<0.05$, respectively). The decrease in the LF region was even larger than in the HF region as expressed by the changes of LF and HF in normalised units. Mean SBP did not change, nor did the spectral content of BPV. SpBRS showed a significant decrease ($p<0.05$).

Atropine markedly reduced the LE of HRV, while ApEn again showed an increase (both $p<0.05$). Also the 1/f slope deviated more from -1 and the DFA index differed more from 1 ($p=0.07$ for DFAα1). In BPV an increase in LE was observed, together with an increase in DFAα2. Also the proportion of CD differing from the surrogate data sets increased from 0% to 100%.

Discussion

Our results support in part the hypothesis that disturbing the autonomic nervous system creates changes in the non-linear modulation of heart rate and blood pressure, but in a more complicated way than we first anticipated. A clear difference was found for scaling and complexity measures and HRV and BPV are affected in different ways. Our results indicate an important contribution of the α-adrenergic system and the vagal nervous system in the generation and control of non-linear heart rate and blood pressure fluctuations. In rats, the β-adrenergic pathways seem to have only a negligible contribution to the non-linear dynamical control. We have found a difference in the effect of autonomic blockade on non-linear indices describing the scaling properties of the time series and the complexity measures of the system. While the cholinergic interventions mostly affected the complexity measures, α-adrenergic changes mostly produced changes in the scaling properties.

The effects of pharmacological blockade on linear HRV and BPV indices are well known and are reproduced by our findings. We will therefore limit the discussion to the non-linear indices.

Vagal nervous system and baroreflex contribution to non-linear dynamics: Cholinergic blockade decreased the non-linear complexity measures of HRV significantly. The non-linear scaling properties did not show a significant evolution. Complexity measures of blood pressure fluctuations during cholinergic blockade clearly showed a higher amount of non-linearity. In all files the CD was found to be attributed to non-linear fluctuations after comparison to the surrogate data. Cholinergic influences are mostly directed to the heart because of the presence of muscarinic receptors. These results suggest that the vagal modulation is mostly responsible for introducing complexity in heart rate variations. The dysfunction of the baroreflex system during cholinergic blockade suggests that the increase in

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non-linear BPV complexity originates either from a feedback mechanism, other than the baroreflex, to compensate for the absence of the baroreflex loop; or from a decreased binding of acetylcholine on muscarinic receptors of the smooth muscle and the endothelium of the vasculature. Such a feedback mechanism might be related to the positive feedback mechanism described by Pagani et al[26] and Legramante et al[27]. In their studies they established a sympathetic connection to the positive feedback reflex. Following their theory, a decrease in the BRS would lead to an augmentation of the positive feedback mechanism. This positive feedback mechanism might play an important role in the increased complexity of BPV during the decreased baroreflex sensitivity.

α-adrenergic contribution to non-linear dynamics: α-blockade showed a decrease in non-linear scaling properties of heart rate variations. The complexity measures were not affected by this intervention. In BPV, on the contrary, the scaling indices suggest an increase in non-linear behaviour. Again, no effect was seen on the complexity measures. α-adrenergic receptors are mostly concentrated in the vascular system and to a lesser extent in the heart. The sympathetic nerve catecholamines preferentially bind to the β-adrenoceptors in the heart, while in the blood vessels they preferentially bind to α-adrenoceptors. Therefore, it appears that the α-adrenergic system acts inhibitory to the non-linear scaling properties of blood pressure variations. The intact baroreflex can be responsible for the unchanged complexity measures. Feedback through this baroreflex of the increased non-linearity in BPV to the heart might contribute to the observed decrease in scaling properties of HRV.

β-adrenergic contribution to non-linear dynamics: Our results of β-blockade on non-linear HRV demonstrate no explicit changes in non-linear behaviour. Only the value of ApEn is increased, but this is also true during α- and cholinergic blockade. Also, on BPV effects were minor: DFAα2 was closer to 1 and the 1/f slope increased. These changes indicate opposite effects on non-linear dynamics and thus it seems that the β-adrenergic system has little involvement in the generation of non-linear dynamics in HRV and BPV in rats.

Only a few previous studies have tried to assess the nature of non-linear fluctuations in animals.

Zwiener et al[28] calculated the correlation dimension and the Lyapunov exponent in rabbits using autonomic blockade, however, the blockade was administered immediately after anaesthesia (ketamine and xylacaine), which influences cardiovascular control and decreases autonomic modulation (own observation in sheep experiments, data not shown). Despite this, autonomic cholinergic blockade decreased the non-linear content of the time series even further. No direct information on the difference with the surrogate data sets was provided. It cannot be excluded that the anaesthesia also influenced the consecutive effects of the autonomic blockades. In our study at least 2 hours were allowed for recovery and each time blood gases were checked to assure respiratory acidosis.

Autonomic blockade in mice[5,29] has demonstrated that their autonomic control differs from humans, making mice less suitable to explore on this perspective. These experiments, however, did prove that it was possible to provoke a higher degree of non-linearity in combination with a decreased linear behaviour. The same holds true for our findings: although the linear variability of BPV was depressed after cholinergic and α-blockade we still found a higher degree of non-linearity. This demonstrates that complexity should not be mistaken for variability.

In dogs, no change in non-linear behaviour was observed after propranolol administration, while additional atropine initiated a clear reduction in non-linear scaling dynamics[30]. Zwiener et al[31] also used an additive blockade protocol in piglets after ketamine sedation, but they first administered cholinergic blockade followed by beta-blockade. They reported a decrease in complexity measures of HRV after cholinergic blockade, while the Lyapunov exponent of the BPV remained unchanged. No additional changes were observed with β-blockade.

Non-linear dynamics in rat cardiovascular control was first studies by Dabiré et al[32]. They suggested an involvement of the α-sympathetic and vagal nerves in the generation of non-linear fluctuations, based on observations of the recurrence plot. A decrease in non-linear HRV behaviour was described after atropine administration and after prazosin administration. Non-linear BPV was only affected (decreased) by prazosin. No changes were found after β-blockade. These results were later confirmed by the same group[33] in hypertensive rats. Skinner et al[34] discovered reduced dimensionality of HRV in rats after ketamine anaesthesia in combination with hemorrhage. Gonzales et al[35] also studied non-linear control of HRV and BPV in rats. They described no effects of β-adrenergic blockade on non-linear HRV and BPV. After α-blockade an increase in non-linearity of BPV was reported. Atropine produced a decrease in non-linear HRV, but not in BPV. In their study the respiratory component of heart rate and blood pressure fluctuations was mathematically suppressed. (frequencies >0.9 Hz).

An intriguing result was that non-linearity of HRV was always associated with tachycardia. Linear HRV indices have been found to depend on heart rate[36]. However, in baseline condition the correlation of non-linear indices and HRV was almost non-existent (only the correlation between the LE and mean RR was significant: $r=0.69$; $p<0.01$; Pearson correlation coefficient).

Physiology behind non-linearity: scaling versus complexity: One consistent finding in these experiments and in our results is the lack of response of both non-linear HRV and BPV after β-blockade, making a strong case against β-adrenergic involvement in the control of non-linear fluctuations. Although β-blockade creates a disturbance in the normal sympatho-vagal equilibrium,

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no changes are observed in the non-linear control of heart rate and blood pressure. Also the effects of atropine on non-linear HRV are clear; a reduction in non-linear heart rate fluctuations is reported in all studies, except for mice, which is in agreement with our results. Atropine reduces the complexity behaviour of heart rate fluctuations. This is supportive for a vagal role in the generation of these fluctuations. At the same time the complexity of blood pressure variations is increased. This might be a compensatory mechanism because of the failure of the baroreflex mechanism, possibly by a sympathetic feedforward reflex. During α-blockade the baroreflex mechanism is intact, and no changes are observed in complexity of both HRV and BPV. α-Adrenergic blockade, however, increases the scaling behaviour of BPV. The intact BRS can play a role in the compensatory decrease that is observed in HRV scaling behaviour.

One of the reasons of conflicting findings for α-adrenergic blockade in literature might be the difference between selective and non-selective blocking agents (Prozasin acting on α1-receptors and Phentolamine acting on both α1 and α2 receptors). In order to describe these mechanisms in more detail new experiments are needed specifically aimed at the different types of receptors. One must also keep in mind that fluctuations are not due to one branch of the autonomic nervous system, but they are the result of a complex interplay and imbalance (in case of autonomic blockade) between the vagal and sympathetic control. This would also benefit the application in human subjects of these methods and would help to explain the use of non-linear dynamics in different kinds of pathologies.

Conclusions

Our data clearly indicated that the α-adrenergic and the cholinergic system are involved in the generation and control of non-linear cardiovascular fluctuations in rats. We showed that non-linear fluctuations are influenced by changing autonomic cardiovascular control and that scaling behaviour is controlled by α-adrenergic pathways and the complexity measures describe the influence of the cholinergic system in which the baroreflex system also plays an important role. There seems to be little involvement of the β-adrenergic system in non-linear cardiovascular control.

Although the number of rats used was rather small in this study, our results are in agreement with previous studies. In addition, they still expand the knowledge about the physiological meaning of non-linear indices of HRV and BPV.

Non-linear signal processing should be based on several methods. Each parameter describes another part of the non-linear content and is clearly affected in different ways. They should best be used in combination with and not instead of linear indices. The choice of the non-linear indices will have to be considered in view of the type of situation or pathology.

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