Cardiorespiratory Coordination in Rats is Influenced by Autonomic Blockade

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Abstract — **Autonomic disturbance creates changes in the modulation of heart rate. In this study we analyzed the influence of sympathetic and vagal blockade on the interaction between cardiac and respiratory rhythms. In seven anaesthetized rats, electrocardiogram (ECG), and respiratory rate were recorded continuously before and after autonomic blockade with either methyl-scopolamine or atenolol. For the assessment of cardiorespiratory coordination, we analyzed the phase locking between heart rate, computed from the R-R intervals of body surface ECG, and respiratory rate, computed from impedance changes, using Hilbert transform. The procedure was carried out for different m:n coordination ratios where, m, is the number of heart beats and n, is the number of respiratory cycles. The changes in percentage of synchronization and duration of synchronized epochs before and after injection were assessed with one-way ANOVA. Sympathetic blockade with atenolol caused an increase (baseline: 0.49 ± 0.03s vs. blockade: 0.54 ± 0.06s) and vagal blockade with methyl-scopolamine caused a decrease (baseline: 0.49 ± 0.03s vs. blockade: 0.45 ± 0.08s) in the duration of synchronized epochs. Neither the overall percentage of synchronized epochs, (baseline: 10.76 ± 3.5% vs. blockade 9.44 ± 4.3%), nor the average locking ratio, 3:1, was significantly affected by autonomic blockade. In conclusion, the phase-locking between heart rhythm and respiration is modulated by both vagal and sympathetic efferences, in the opposite directions.**

Keywords — **sympathetic nervous system, vagal blockade, respiration, heart rate**

I. Introduction

The interaction between cardiac and respiratory oscillations can be studied through synchronization of these oscillators. Several studies have been undertaken on cardiorespiratory synchronization not only for subjects at rest [1,2,3], but also for subjects under the influence of anesthesia and drugs [4,5]. Synchronization is proven to be an intrinsic cardio-respiratory phenomenon and not just an artifact [6]. However, little is known about the physiological basis and the underlying mechanism responsible for the cardiorespiratory interaction.

The parasympathetic (vagal) system is known to have a great influence on heart rate [5] and heart rate variability [7], but its effect on cardiorespiratory coordination is yet to be revealed.

In this study we investigated the effect of sympathetic and vagal blockade on cardiorespiratory coordination in rats.

II. Methods

A. Animal preparation

Seven male Wistar Hooded rats, weighing 250-300 g (obtained from Flinders University, Bedford Park, South Australia), were used for this study. Experiments were conducted in accordance with the European Community Council Directive of 24 November 1986 (86/609/EEC), and were approved by the Flinders University Animal Welfare Committee. During the experiment, ECG, respiratory rate and blood pressure were recorded continuously before and after autonomic blockade with either methylscopolamine or atenolol. The signals were acquired using MacLab interface and Chart software (ADInstruments, Sydney, Australia).

B. Data analysis

Pre-processing: Custom made computer software developed under MATLAB® was used to extract the R-peaks from the recorded ECG signal using parabolic fitting. The RR intervals obtained from the time-points of the R-peaks were scanned for artifacts and, if necessary, manually edited. In order to obtain baseline values, we selected a 40 minute interval, starting 10 minutes prior to the blockade. For the analysis of synchronization during blockade, we selected 30 minute epochs each after vagal blockade with methyl-scopolamine and sympathetic nervous system blockade with atenolol, starting 5 minutes after the injection.

Respiration analysis: Respiratory signals consist of noisy, linear, nonlinear and non-stationary components. To extract the essential respiratory rhythm related components from the signal, we used empirical mode decomposition (EMD). EMD separates data into non-overlapping time-scale components. The multi-component signal is decomposed into a series of intrinsic mode functions (IMF) where each IMF represents a simple oscillatory mode embedded in the data [8]. The IMF that best matched the respiratory rhythm was selected for further analysis.

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In order to determine the maximum and minimum points of each respiratory cycle, known as maxima and minima respectively, we calculated the derivative of the respiratory signal and considered those points that were equal to or very close to zero (window length: 0 - 0.2 mV). The average of the time series obtained from minima-to-minima intervals was used to calculate the average respiratory period.

Synchronization analysis: Synchronization between two periodic oscillators can be defined as the appearance of some relationship in the form of locking of their phases or adjustment of rhythms. We used Hilbert transform to calculate the phases of the cardiac and respiratory signals. If we denote the phase of heartbeat as Φ_c and respiratory signal as Φ_r and considering that the cardiovascular system completes m heartbeats in n respiratory cycles, then the condition for phase locking can be given as

$$\left|m\Phi_c - n\Phi_r\right| \le const \quad (1)$$

In other words, if the phase difference between the two oscillators was within a certain threshold value and remained stable for n respiratory cycles, the oscillators were considered synchronized. The cardiorespiratory synchrogram, CRS, provide a visual tool to detect synchronization. If t_k is the time of the kth R-peak, then the normalized relative phase of heart beat is

$$\Psi_n = \frac{1}{2\pi}\left(\phi(t_k) \bmod 2\pi m\right) \quad (2)$$

CRS is generated by plotting Ψ_n against t_k which, in case of m:n synchronization, results in m horizontal lines. In order to determine the values of m and n, we selected one value of n at a time and looked for coordinations at different values of m. The study was carried for the following m:n coordinations: n = 1: m = 2,…,7 and n = 2: m = 5,7,9,11,13. We used a threshold value of 0.035 for the phase difference as it was suggested by Cysarz et al. [9].

Statistical analysis: Statistical analysis was performed with GraphPad Prism® version 5.0. The differences before and after autonomic blockade were analyzed by one-way analysis of variance (ANOVA) for multiple measurements and Wilcoxon test for paired comparisons. Data are expressed as the mean±SD. For the analysis, $p < 0.05$ was considered statistically significant.

III. Results

A. Effect of autonomic blockade on heart rate

Methyl-scopolamine, which was used to block the parasympathetic nervous system, caused an increase in heart rate, while atenolol, used to block the sympathetic nervous system, caused a decrease in heart rate (Fig. 1). The differences were statistically significant, $p < 0.01$, and are summarized in Tab.1.

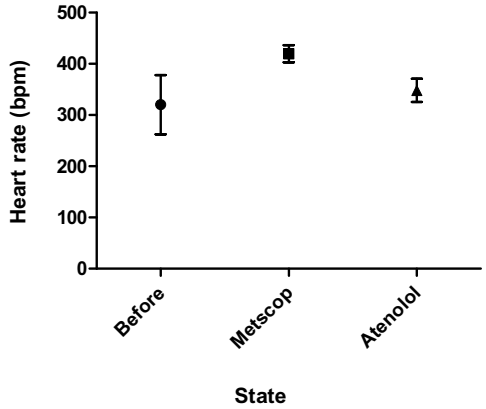

Fig. 1 Mean±SD of heart rate (beats per minute) obtained from seven rats prior and after injection of methyl-scopolamine (metscop) and atenolol

B. Effect of autonomic blockade on respiratory frequency

There was no significant effect (Fig. 2) of either atenolol or methyl-scopolamine on the respiratory frequency.

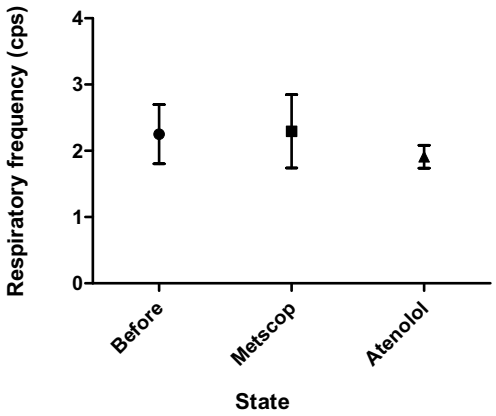

Fig. 2 Mean±SD of respiratory frequency (cycles per second) obtained from seven rats prior and after injection of methyl-scopolamine (metscop) and atenolol

C. Effect of autonomic blockade on average duration of synchronized epochs

The average duration of synchronized epochs was longer (Fig. 3) after injecting with atenolol (baseline: 0.49 ± 0.03s

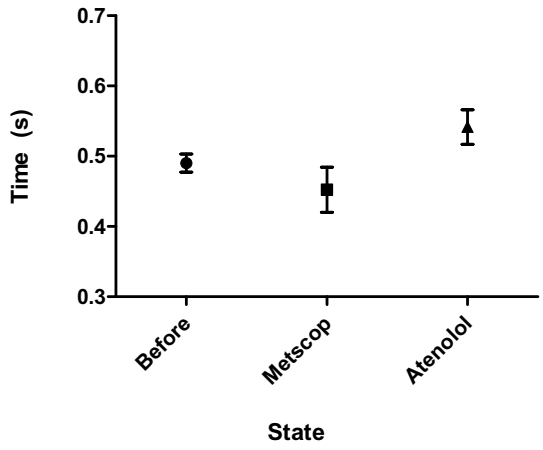

Fig. 3 Mean±SD of duration of synchronized epochs obtained from seven rats prior and after injection of methyl-scopolamine (metscop) and atenolol.

vs blockade: 0.54 ± 0.06s). On the other hand, injection of methylscopolamine caused a decrease in the average duration of synchronized epochs (baseline: 0.49 ± 0.03s vs blockade: 0.45 ± 0.08s). These effects were statistically significant.

D. Effect of autonomic blockade on percentage of synchronization

Vagal blockade caused a decrease while sympathetic blockade caused an increase in percentage of synchronization in six rats. In one rat, the percentage of synchronization was higher after vagal blockade. The results were not statistically significant. However, percentage of synchronization followed a similar trend (Fig. 4) after vagal blockade showing significant results in six rats.

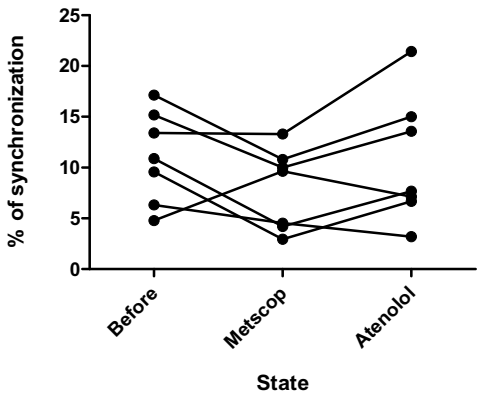

Fig. 4 Percentage of synchronization in seven rats prior and after injection of methyl-scopolamine (metscop) and atenolol.

Table 1 Total recording time (TRT), heart rate (HR), RR interval (RRint), respiratory frequency (RespFr), percentage of synchronization (PofSync) and duration of synchronized epochs (SyncDur) in seven rats represented as mean±SD as well as p-values of Wilcoxon test for paired comparisons

	Before	Metscop	Atenolol	p
TRT (min)	40.2 ± 4.5	30.5 ± 5.1	29.7 ± 4.3	-
HR (bpm)	320.1 ± 27.6	419.5 ± 16.9	347.9 ± 22.7	0.01
RRint (s)	0.18 ± 0.01	0.14 ± 0.01	0.17 ± 0.01	0.01
RespFr (cps)	2.25 ± 0.45	2.29 ± 0.56	1.91 ± 0.17	0.69
Pof Sync	11.04 ± 4.5	7.78 ± 4.1	10.81 ± 6.1	0.08
SyncDur (s)	0.49 ± 0.03	0.45 ± 0.08	0.54 ± 0.06	0.03

E. Effect of autonomic blockade on the average locking ratio

The cardiorespiratory coordination occurred for the phase-locking ratios of 2:1, 3:1, 4:1, 5:2 and 7:2. The 3:1 phase-locked state was frequently observed and was not significantly affected by autonomic blockade.

IV. Discussions

This is the first study to investigate the effect of autonomic blockade on cardiorespiratory coordination. Both sympathetic as well as vagal efferents affect cardiorespiratory coordination with opposite directions.

Our results show that autonomic blockade produces changes in heart rate. These findings are in accordance with the results reported earlier in the literature [5,7,10]. Contrary, no changes in the respiratory rate were observed. Consequently, heart rate rather than respiratory rate seems to be the predominant factor causing the phenomenon of phase locking between heart rate and respiration.

The reduction of cardiorespiratory coupling after vagal blockade is in line with the observation that vagal outflow to the sinus node of the heart is the predominant driver of respiratory sinus arrhythmia.

The increase of cardiorespiratory coordination after sympathetic blockade was unexpected. Possibly, sympathetic effects on heart rate disturb the respiratory sinus rhythm and a sympathetic blockade consequently increases the amount of observed phase locking between heart rate and respiration. Although the average duration of synchronized epochs and percentage of synchronization was found to increase with sympathetic blockade and decrease with vagal blockade, the change in percentage of synchronization did not

reach statistical significance. This is because one of the rats showed an opposite behavior compared to others, (see Fig. 4) whose results would otherwise considered being statistically significant.

V. Conclusions

Vagal and sympathetic blockade cause a respective decrease and increase in the duration of synchronized epochs and percentage of synchronization between heart rate and respiration in rats.

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