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Research Article

Changes In Heart Rate and Short Term Heart Rate Variability with Respect to the Circadian Pattern In Beta-Adrenoceptor 1, 2, and 3 Knockout Mice

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Abstract

Objective: Aim of the present study was to evaluate changes in heartrate and short term heartrate variability (HRV) with respect to the circadian rhythm in total beta-adrenoceptor 1, 2, and 3 knockout mice (TBKO) compared to wildtype controls (WT).

Methods: Telemetric electrocardiographic recordings were performed (n=28) and HRV was calculated using time domain and frequency domain analysis. Holter-ECG data were analyzed in the morning (7 a.m.), in the afternoon (5 p.m.) and at night (2 a.m.), in order to investigate the circadian pattern.

Results: Heart rate was significantly lower in KO mice. Differences in HR (TBKO 474 ± 51 min^{-1}; WT 513 ± 45 min^{-1}; p=0.05) were predominantly obtained in the morning hours. With respect to HRV, time and frequency domain analyses demonstrated a decrease in the standard deviation of all NN intervals (SDNN), total power, and frequency ranges in TBKO mice. Further, the low frequency / high frequency (LF/HF) ratio, indicating predominantly vagal activity, was lower in TBKO mice. Especially in the morning hours we this difference was prominent (WT 1.21 ± 0.43, WT 1.00 ± 0.48; p=xy), whereas, HR and HRV demonstrated no significant alterations in circadian pattern.

Conclusion: Lack of sympathetic beta-adrenergic stimulation resulted in decreased HRV and increased vagal activity in TBKO mice. Accordingly, our data show that sympathetic beta-adrenergic stimulation elevates HRV and is responsible for its circadian variation.

Keywords: Heart Rate Variability; Circadian Pattern; Total Beta Knockout Mice

Abbreviations

ANS: Autonomic Nervous System;

eNOS: Endotheliale Stickoxidsynthase;

FFT: Fast-Fourier-Transformation;

HF: High Frequency;

HR: Heart Rate;

HRV: Heart Rate Variability;

TBKO: Total Beta-Adrenoceptor Knockout Mice;

LF: Low Frequency;

LF/HF Ratio: Ratio of Low Frequency to High Frequency;

NN: Normal to Count;

SDNN: Standard Deviation of all NN Intervals;

VLF: Very Low Frequency;

WT: Wild Type Mice

Introduction

Heart rate variability (HRV) is used as a non-invasive method to analyse autonomic nervous system (ANS) activity and to asses the balance of sympatho-vagal activity on the heart. Decreased HRV is associated with an increased risk of ventricular arrhythmias and has been shown to constitute an independent prognostic factor for mortality in patients after acute myocardial infarction [1]. HRV is affected by a complex interaction of the components of the ANS; the sympathetic nervous system causes an increase, the parasympathetic nervous system a decrease of the HRV [2].

The ANS exerts its influence through various receptors. In this study, we focus on the beta-adrenoceptors which are essential in the regulation of the HR and HRV. There are three sub-types of these adrenoreceptors: beta1, beta2, and beta3. The existence of a further sub-type, beta4 [3], is postulated, showing similarity to the beta1-adrenoreceptor [4].

Until now, the role of beta-adrenoreceptors in the regulation of HR has been examined with the help of specific agonists and antagonists. But even using highly selective pharmacological methods, it is extremely difficult to block specific receptor subtypes in vivo over time. Chronic administration of a beta-adrenoreceptor blocker causes an up-regulation of beta-adrenoreceptors and consequently frustrates the ability to investigate their specific role [5].

The investigation of total beta receptor (1, 2, and 3) knockout mice (TBKO) represents an important addition to standard pharmacological methods of studying the physiological value of these receptors.

Methods

The experiments were performed in 12 adult TBKO mice (10 male, 6 months old, C57bl6 background) and 20 adult wild type (WT) control mice (female, 5 months old, C57bl6). All mice were housed in a laboratory and exposed to a natural day-night rhythm.

Surgery

The mice received general anaesthesia with Isoflurane during the implantation of a radiotelemetric transmitter (weight approx. 1 g, volume < 1 cm^3) in a subcutaneous cavity between the scapulae. The electrode cables were tunnelled subcutaneously under the forelegs to the chest wall allowing a permanent subcutaneous ECG recording. To reduce postoperative pain and the risk of infection, the mice were treated with Metamizol and Cefuroxime. On the third postoperative day continuous 24 h ECG recordings were performed and analysed offline. For recording, the Data Science- (Transmitter/Receiver) and Power Lab- (AD Instruments, Milford, MA, USA) systems were utilized. For analyses, the commercially available software application HRV (AD Instruments, Milford, MA, USA) was used.

Complex analyses of HR and HRV

The program detected all signals that exceeded a certain voltage (adjusted to the individual ECG) as R wave of a QRS complex. All intervals generated by the sinus node were called NN (normal to count). All NN intervals < 60 ms or > 350 ms were considered as artefacts and all NN intervals < 100 ms or > 300 ms as ectopics. Both were excluded from the analysis. Before processing HRV, a manual review of all NN intervals was undertaken to correct false or unrecognized beats. Periods of 10 – 15 min were analysed three times a day, at 7 a.m., 5 p.m., 2 a.m. (+/- 1 hour). At these times the HR und HRV were measured. To quantify HRV, time as well as frequency domain analysis were performed.

One of the parameters of the time domain analysis is HR. Another parameter is the standard deviation of all NN intervals (SDNN). This parameter is based on the direct measurement of the temporal NN-distances. The SDNN (ms) reflects the cyclical influences over the recorded period (ideally 24 hours) and is thus a marker of the total variability. Because the SDNN is influenced by the recording period, a standardized recording length of 10 – 15 min is set.

Conversion of the periodic oscillations of the HR from time domain into frequency domain parameters was performed using Fast-Fourier-Transformation (FFT). From the continuous changes, different frequency ranges occur with multi-peaked spectra.

Three main spectral components are distinguished in a spec-

trum: very low frequency (VLF) 0.003-0.04 Hz, low frequency (LF) 0.04-0.15 Hz and high frequency (HF) 0.15-0.4 Hz [6]. The autonomic balance of HRV is reflected especially in the LF and HF range. The LF range provides information about sympathetic and vagal activity; the HF range reflects vagal activity [6].

We processed the VLF component by using the normalized parameter of HF and LF power (nu – normalized units), which represent the relative value of each power component in proportion to the Total Power minus the VLF component. The representation of LF and HF in normalised units emphasizes the controlled and balanced nature of the two components of the ANS. Moreover, the normalisation tends to minimize the effect of the changes in Total Power on the values of LF and HF components. The ratio of low frequency to high frequency (LF/HF Ratio) is a parameter to describe the sympathovagal balance [7]. An increase of this parameter implies a shift towards sympathetic, a decrease towards parasympathetic activity [8].

The Total Power reflects the variance of all NN intervals and the sum of all the energy densities and thus reflects a marker of the total variability.

Statistical analysis

The measured data are presented as mean ± standard deviation. Comparisons between groups were analyzed statistically with the two-sided, unpaired Student's T-Test. A value of $p < 0.05$ was considered statistically significant.

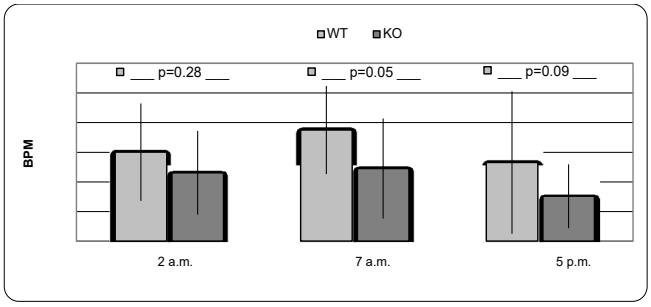
Results and Discussion

The analysis of HRV is a non-invasive method of analysing and recording the activity of the ANS and is used to evaluate the sympatho-vagal balance. This study investigated HRV of total beta-adrenoceptor knockout and wild type mice. We examined the circadian pattern of each species and the differences between the two species. Using short-term ECG recordings at three different times of day, various parameters of HRV were explored. Our data analysis was performed in accordance to the latest recommendations [6]. Only few studies of the HR and HRV performed on genetically modified mice in which one or more beta-adrenoreceptors have been knocked out [5]. This is the first study investigating HR and HRV in TBKO mice.

Heart rate

A comparison of HR between the two groups in our study showed that HR of KO mice was lower than that of WT irrespective of the time of the day. Significant differences of the HR were obtained predominantly in the morning. Interestingly, TBKO mice demonstrated no alteration in the circadian pattern of the HR. The highest absolute HR was obtained in the morning (WT 513 ± 45 BPM, TBKO 474 ± 51 BPM, p=0.07), the lowest in the afternoon (WT 475 ± 72 BPM, TBKO 455 ± 32 BPM, p=0.06). See *figure 1* for further details. The reduced HR in the KO mice reflects the absence of the positive chronotropic effect of stimulated beta-adrenoreceptors.

Figure 1. Inter-specific comparison of both mouse groups at the different times of day with respect to the HR, as a parameter of time domain HRV parameters.



BPM: beats per minute
WT: wild type mice
KO: beta-adrenoceptor 1, 2, and 3 knockout mice

A significant difference of HR was demonstrated only in the morning. At this time both groups showed an increased HR. This seems to be based on other control mechanisms. There is, for example, a morning increase in various hormone levels, such as Cortisol which is a "stress hormone" and causes an increased HR.

Sapoznikov et al. (1992) observed an increase in sympathetic activity on awakening in humans. The data collected in our study showed an increased HR in the morning in both groups of mice, as an indication for increase sympathetic activity. But this was more obvious in the WT mice. Perhaps this illustrates the lack of response to this increased sympathetic activity in beta-receptor 1, 2, and 3 knock-out mice.

Heart rate variability

To quantify HRV, two different analyses were used: the time and the frequency domain analysis.

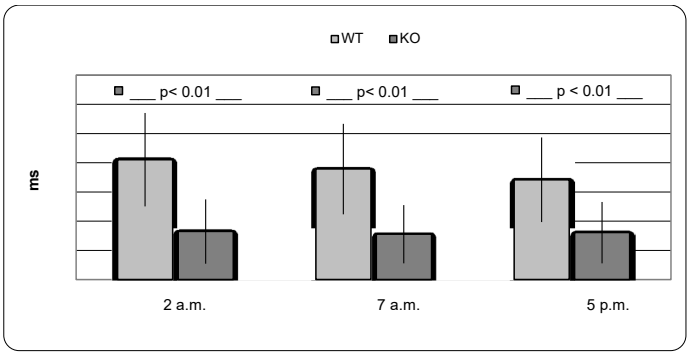
As one of the time domain parameters we focussed on SDNN.

The SDNN demonstrated significant differences between the two groups. The knockout mice showed a decrease SDNN, in comparison to WT. At night we could find the highest value of SDNN in both groups of mice (WT 10 ± 3 ms, KO 5 ± 2 ms). A significant circadian pattern in either group could not be unmasked. Figure 2 demonstrates this inter-specific comparison of both groups.

The frequency domain analysis demonstrated highly significant differences in the frequency ranges between the two groups. The Total Power is clearly reduced in KO. The circadian

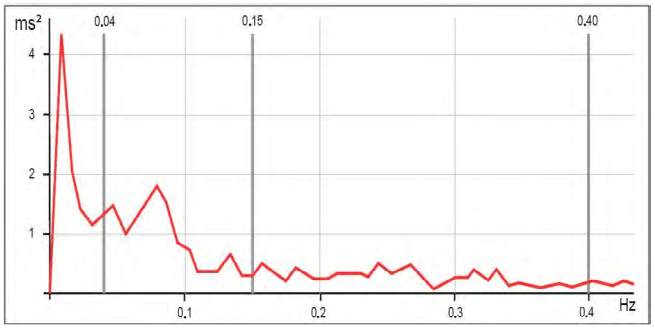
pattern is less clearly apparent. The highest values we detected at night (WT 88.5 ± 60.6 ms^2, KO 30.2 ± 21.8 ms^2), when the vagal activity seems to be important for the HRV.

Figure 2. Inter-specific comparison of both mouse groups at the different times of day with respect to the SDNN, as a parameter of time domain HRV parameters.



WT: wild type mice
KO: beta-adrenoceptor 1, 2, and 3 knockout mice

Figure 3. Example of a Power Spectrum from a WT.



WT: wild type mice

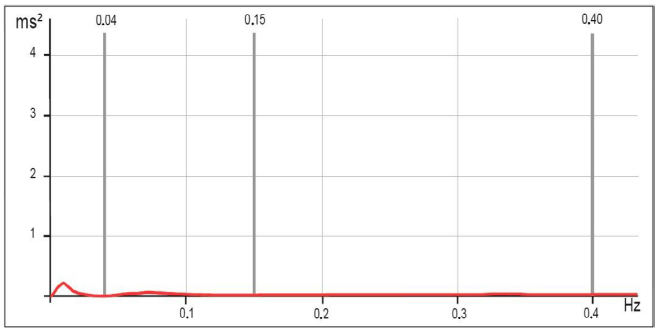
The different frequency components in the KO mice are reduced. Even with these parameters, the highest values were measured at night. Consider, for example, the LF in normalizes units, we measured at night 31.5 ± 7.6 ms^2 in the group of WT and 4.7 ± 4.7 ms^2 in the group of KO. We could find the same situation in HF in normalizes units (WT 26.5 ± 7.2 ms^2, KO 3.8 ± 3.4 ms^2). The circadian pattern is altered by the lack of influence of beta-adrenoreceptor 1, 2, and 3, but differences between the groups are not significant.

The LF/HF ratio is a parameter to describe the sympatho-vagal balance [7]. An increase in this ratio implies a shift towards sympathetic activity, a decrease, parasympathetic [8].

Our LF/HF ratio values were lower in the KO mice group, for example in the morning we could find this difference (WT 1.21 ± 0.43, WT 1.00 ± 0.48). But the difference between the groups was not significant. Table 1, 2, and 3 demonstrates the values of the inter- and intra-specific comparison of the different parameters of the frequency domain analyse.

As Calvert 1998 postulated, the lower LF/HF ratio values in KO reflect a shift of the sympatho-vagal balance towards parasympathetic activity. It was not possible to completely eliminate sympathetic influence in the present study because in our mice the beta 4-adrenoreceptor was not "knocked out" (a degree of sympathetic activity may still be present).

Figure 4. Example of a Power Spectrum of a KO.



KO: beta-adrenoceptor 1, 2, and 3 knockout mice

Table 1. Intra-specific comparison of the WT at the different times of day with respect to the frequency domain HRV parameters (**: p<0.01 for WT for each time; *: p<0.05 for WT for each time)

	WT 2 a.m.	WT 7 a.m.	WT 5 p.m.
Total Power (ms²)	88.5 ± 60.6	74.8 ± 46.0	65.4 ± 37.0
VLF (ms²)	30.5 ± 23.0	22.0 ± 16.8	20.3 ± 17.4
LF (ms²)	17.1 ± 14.0	15.9 ± 10.2	14.0 ± 8.9
LF normalized (nu)	31.5 ± 7.6	31.0 ± 8.8	20.0 ± 11.9
HF (ms²)	14.9 ± 14.9	13.3 ± 6.3	12.9 ± 10.2
HF normalized (nu)	26.5 ± 7.2	27.0 ± 6.7	28.0 ± 11.0
LF/HF Ratio	1.25 ± 0.33	1.21 ± 0.43	1.20 ± 0.60

VLF: very low frequency 0.003-0.04 Hz
LF: low frequency 0.04-0.15 Hz
HF: high frequency 0.15-0.4 Hz
WT: wild type mice

With the help of examinations in which a combination of subtype-specific agonist and antagonist are used, there is another possibility to analyse the specific role of the different beta-adrenoreceptor subtypes. This is limited by the various dimensions of the subtype selectivity of these pharmacological agents. Studies with pharmacological beta-adrenoreceptor blockade demonstrate a decreased HR and an increased HRV. The increase in HRV is reflected in a significant increase of Total Power, the HF and LF component. In addition a decrease in the LF/HF Ratio has been shown. Using a pharmacological beta-adrenoreceptor blockade, the normal circadian changes

in LF and HF power and LF/HF Ratio are eliminated.

Table 2. Intra-specific comparison of the KO at the different times of day with respect to the frequency domain HRV parameters (**: p<0.01 for WT for each time; *: p<0.05 for WT for each time)

	KO 2 a.m.	KO 7 a.m.	KO 5 p.m.
Total Power (ms²)	30.2 ± 21.8	26.1 ±20.2	29.7 ± 25.3
VLF (ms²)	1.5 ± 1.4	1,1 ± 1.6	2.0 ± 2.2
LF (ms²)	0.9 ± 1.1	0.5 ± 0.4	1.4 ± 1.3
LF normalized (nu)	4.7 ± 4.7	5.6 ± 5.0	7.9 ± 7.0
HF (ms²)	0.6 ± 0.6	0.5 ± 0.3	1.5 ± 1.5
HF normalized (nu)	3.8 ± 3.4	5.8 ± 5.2	7.4 ± 4.3 **
LF/HF Ratio	1.13 ± 0.48 **	1.00 ± 0.48	1.05 ± 0.94

VLF: very low frequency 0.003-0.04 Hz
LF: low frequency 0.04-0.15 Hz
HF: high frequency 0.15-0.4 Hz
KO: beta-adrenoceptor 1, 2, and 3 knockout mice

Table 3. Inter-specific comparison of both mouse groups at the different times of day with respect to the frequency domain HRV parameters (**: p<0.01 for WT for each time; *: p<0.05 for WT for each time)

	WT 2 a.m.	KO 2 a.m.	WT 7 a.m.	KO 7 a.m.	WT 5 p.m.	KO 5 p.m.
Total Power (ms²)	88.5 ± 60.6 **	30.2 ± 21.8 **	74.8 ± 46.0 **	26.1 ± 20.2 **	65.4 ± 37.0 **	29.7 ± 25.3 **
VLF (ms²)	30.5 ± 23.0 **	1.5 ± 1.4 **	22.0 ± 16.8 **	1,1 ± 1.6 **	20.3 ± 17.4 **	2.0 ± 2.2 **
LF (ms²)	17.1 ± 14.0 **	0.9 ± 1.1 **	15.9 ± 10.2 **	0.5 ± 0.4 **	14.0 ± 8.9 **	1.4 ± 1.3 **
LF normalized (nu)	31.5 ± 7.6 **	4.7 ± 4.7 **	31.0 ± 8.8 **	5.6 ± 5.0 **	20.0 ± 11.9 **	7.9 ± 7.0 **
HF (ms²)	14.9 ± 14.9 **	0.6 ± 0.6 **	13.3 ± 6.3 **	0.5 ± 0.3 **	12.9 ± 10.2 **	1.5 ± 1.5 **
HF normalized (nu)	26.5 ± 7.2 **	3.8 ± 3.4 **	27.0 ± 6.7 **	5.8 ± 5.2 **	28.0 ± 11.0 **	7.4 ± 4.3 **
LF/HF Ratio	1.25 ± 0.33	1.13 ± 0.48	1.21 ± 0.43	1.00 ± 0.48	1.20 ± 0.60	1.05 ± 0.94

VLF: very low frequency 0.003-0.04 Hz
LF: low frequency 0.04-0.15 Hz
HF: high frequency 0.15-0.4 Hz
KO: beta-adrenoceptor 1, 2, and 3 knockout mice

The results of our study on beta 1, 2, and 3 KO mice, showing a decrease in HR and LF/HR ratio, agree with data collected from humans, that there is a shift of the sympatho-vagal balance toward vagal influence [10].

In their work with rats, Kuwaharam et al. [11] describe a significant decrease of LF power by sympathetic blockade with Propanolol (blockade of beta1, and 2 adrenoreceptors). A significant effect on the HF power is not described.

Pereira-Junior et al. [12] also used Propanolol and additional parasympathetic blockade with Atropine. HR was significantly decreased in the presence of the muscarinic antagonist Atropine, and increased in the presence of the beta-adrenoreceptor antagonist propranolol**.** The HRV was also altered. The LF power was significantly decreased in the presence of Propranolol and HF power was significantly decreased in the presence of atropine. In these experiments, no implants transmitters were used, instead of this custom-made elastic cotton jackets were used (recording time 5 min) [10].

In our study, we detected a significant decrease of LF and HF power and a shift of LF/HF ratio in the direction of HF power. This decreased LF/HF ratio indicates a greater decrease of LF power than HF power. The LF range provides information about sympathetic and vagal activity; the HF range reflects vagal activity [6]. Because of this, especially the LF ranges are influenced by the lack of sympathetic activity.

Propranolol produces blockade of beta1, and 2 adrenoreceptors with no effect on beta3 adrenoreceptors. In the present study, we have knocked out beta1, 2, and 3 beta adrenoreceptor. Therefore, we can assume an important role of beta3 receptors in the modulation of HF power. He seems to be a mean agent of the sympathetic activity with respect to HRV

Ecker at al. [5] analysed the effect of beta1, beta2, and beta1/beta2 double knockout on HR and HRV in mice. In comparison to the beta1 and beta1/beta2 double knockout mice, WT mice or beta2 knockout mice show a decrease in HR. In beta1 knockout mice an increase in HRV (SDNN as a parameter) and an increase in normalized HF and LF power was observed. Compared with mice with intact beta1-adrenoreceptor, regardless of the presence or absence of the beta2-adrenoreceptor, no increase was seen.

Our results of HR agree with the results of other examinations. We found a decrease of HR in beta 1, 2, and 3 KO mice.

In contrast to Ecker et al. [5], we also found a decrease in HRV. This difference may be related to the additionally knocked out beta3 receptor in our mice and may point to an essential role of this receptor in the modulation of HRV.

In some studies, a stimulation of beta3 adrenoreceptor on eNOS way via the release of NO is described. This causes a negative inotropic effect [13]. It represents a negative feedback mechanism to the beta1 adrenergic stimulation and, thus, could be involved in the increase of HRV by antagonising the beta1 adrenergic induced reduction of HRV.

Evidence exists for the existence of beta4 adrenoreceptor in a human trial [4]. This beta4 adrenoreceptor has similarity to the beta1 adrenoreceptor [14].

In the present study the beta4 adrenoreceptor was not knocked out, so the sympathetic nervous system may have influenced cardiovascular regulation through this receptor. Because this

beta4 adrenoreceptor has functional similarities to the beta1 adrenoreceptor our HR and HRV findings could have been influenced by excessive stimulation of the beta4 adrenoreceptor.

Conclusion

The loss of sympathetic beta-adrenergic stimulation, caused by depletion of beta-adrenoceptor 1, 2, and 3, resulted in decreased HRV.

Comparing WT and KO mice, the latter showed a decreased HRV without circadian pattern.

The results of short term HRV clearly demonstrated a significant change towards vagal activity.

Accordingly, these data show that sympathetic beta-adrenergic stimulation elevates HRV and reflects the basis for its circadian variation.

Circadian rhythmic of HR seemed to be uninfluenced by sympathetic beta-adrenergic stimulation.

Limitation

The present study considered only very short recordings at three different times of day and therefore it is a very limited instrument to analyse the circadian pattern. The interpretation of these results could be erroneous.

Knockout mice usually show absence of the exanimate protein at a very early stage of life. Studies of knockout mice can therefore not be compared directly with those using pharmacologically suppressed sympathetic activity because some of the changes, which are observed in KO mice, result from more complex adaptation mechanisms and may not be a direct result of the absence of the protein.

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