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Original Communication

Chronobiological aspects of reoxygenation impact on the heart rhythm disorders in Wistar rat model

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ABSTRACT

The clinical trials unanimously ascribe the increased risk of cardiac events to apneic episodes. The effect of reoxygenation after apneic episode on the development of ventricular arrhythmias have not been studied in detail as a function of the light-dark cycle (LD cycle). We aimed to study the effect of reoxygenation on ventricular arrhythmia threshold (VAT) with respect to the LD cycle. Experiments were undertaken in female Wistar rats anaesthetised with ketamine/ xylazine (100mg/kg+15mg/kg, i.m.) after adaptation to a 12h:12h LD cycle for 4 weeks. The animals were ventilated artificially using a respiratory pump with the parameters of normal ventilation and reoxygenation as follows: V_T = 1 ml/100 g and respiratory rate, 50 breaths/min. The apneic episode was simulated by switching off the respirator for 2 minutes. The results clearly demonstrate the pro-arrhythmogenic effect of apneic episode regardless of the LD cycle. During reoxygenation, anti-arrhythmogenic effect (increase in VAT) was observed in the dark (active) part in contrast to pro-arrhythmogenic effect (decrease in VAT), which was demostrated during the light (nonactive) part of the day. These data suggest that heart rhythm disorders attributed to apneic episode can be attenuated or amplified by subsequent reoxygenation dependent on synchronization to the LD cycle.

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INTRODUCTION

Circadian rhythm sleep disorders such as jet lag syndrome [1] and shift work sleep disorder [2] are specifically attributed to dysfunctions or insufficiencies in the circadian system (the system controlling the 24-h oscillations of the functions in organisms). Taking into consideration the pre-eminence of the circadian clock in timing sleep, it is likely that other sleep disorders (e.g, insomnia) are also linked to abnormalities in the circadian system [3].

Problems related to the connection of the circadian rhythmicity of cardiovascular events with obstructive or central sleep apnoeas have not been studied in detail. Some clinical trials have stated that acceptance of circadian rhythmicity may be relevant for screening of obstructive sleep apnoea (OSA), and may have value in predicting future cardiovascular events [4, 5].

It has been shown that apneic episodes (obstructive or central type) produce acute systemic asphyxia and increase the risk of heart-rhythm disorders [6-10]. These clinical trials unambiguously ascribe the increased risk of cardiac events to apneic episodes occuring during sleep. However, the effect of the recovery of oxygen delivery (reoxygenation) after apneic episodes on the onset or development of ventricular arrhythmias was not considered. Reoxygenation after apneic episodes does not automatically normalize myocardial properties (electrophysiological and mechanical), but can

also increase the risk of reoxygenation arrhythmias [11, 12]. The rat experimental studies refer to the fact that reoxygenation after hypoventilation changed the ventricular arrhythmia threshold (VAT) circadian rhythm to inverse one with the highest electrical resistance of the rat ventricular myocardium to the onset and development of arrhythmias between 12:00h and 15:00h and highest susceptibility between 24:00h and 03:00h with respect to circadian rhythms during normal ventilation (the highest susceptibility between 12:00h and 15:00h and highest resistance between 24:00h and 03:00h) [13, 14].

Thus, clinical studies suggest a link between sudden death from cardiac causes and OSA dependent on circadian timing [4, 5]. From the clinical point of view, the opened question remains: can patients with OSA, without cardiac diseases, be more susceptible to heart rhythm disorders after a change in synchronisation with local time (shift workers, jet-lag syndrome) and what is possible role of reoxygenation in the development of the heart rhythm disorders in these patients?

In our rat experimental model, we aimed to evaluate the share of the subsequent reoxygenation after apneic episode of the central type in the development of the ventricular arrhythmias during active (dark) and non-active (light) part of the regime day.

METHODS

Ethical approval of the study protocol

The present study conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication number 85-23, revised 1996). The study protocol was also approved by the Ethics Committee of Medical Faculty of Safarik University (number of permission: 2/05).

Anaesthesia and adaptation of animals

The experiments were undertaken in female Wistar rats (weight 300±20g, age, 3-4 months). Rats were anaesthetised using ketamine/xylazine anaesthesia (ketamine 100 mg/kg (Narkamon, Prague, Czech Republic) + xylazine 15 mg/kg (Rometar, Prague, Czech Republic; i.m.). Anaesthesia was maintained such that painful stimuli and surgery did not evoke noticeable motor or cardiovascular responses. Upon completion of the experiments, rats were killed by cardiac administration of an overdose of ketamine.

Rats were adapted to a light-dark (LD) cycle of 12h:12h in a light- and climate-controlled room (relative moisture from 40% to 60%, temperature 24 °C in the cage, 2 animals per cage) for 4 weeks and were allowed access to food and water *ad libitum*. The cleaning of cages was performed each second day at the red light during dark phase of experiment (dark from 06:00h to 18:00h). The effect of the light part of the day on the electrical stability of the heart and heart rate (HR) was evaluated after LD adaptation, with the dark part of day from 18:00h to 06:00h. The experiments were carried out twice (the first animal between 09:00h and 10:00h and the second animal between 12:00h and 13:00h). The effect of the dark part was evaluated after inverse setting of the LD cycle, with the dark part from 06:00h to 18:00h, with the experiment time identical to the light part of the experiment (Scheme 1).

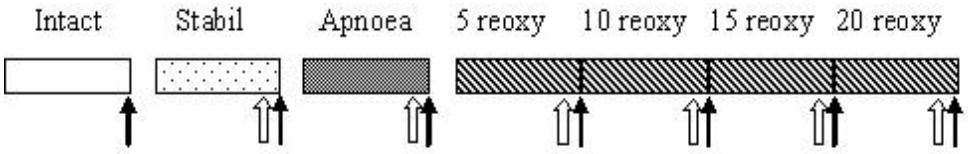
Artificial ventilation

Rats were placed in the supine position on a pre-heated table. The body temperature was maintained on level of the rectal temperature measured before anaesthetic agent application. Heating by an infrared lamp was used to prevent hypothermia effect on the heart rate. The trachea was exposed at the mid-cervical level and cannulated using a plastic tube. A tracheal cannula was attached to a volume-rate-regulated artificial ventilator (UGO Basile, Comerio-Varese, Italy) and animals ventilated by room air. The parameters of the initial ventilation and reoxygenation were a respiratory rate of 50 breaths/min and a tidal volume of 1 ml/100 g body weight. The parameters of ventilation were chosen on the basis of validated methods of artificial controlled ventilation using room air for pentobarbital-anaesthetised rats, which can be applied for the preservation of normal acid-base balance. Apneic episodes were simulated by switching off the ventilator for 2 min.

Experimental protocol

Animals were randomly divided into seven groups (14 animals in each group) for the light and dark

Scheme 1. Adaptation of rats to the light regimen 12h:12h in a special room with constant humidity (50-60%) and constant temperature in cages (24°C) for 4 weeks. The light part of the day is denoted by white stripe, and the dark part of the day by the black stripe. Arrows indicate the time of the running of the experiment: the first arrow at 09:00h and the second arrow at 12:00h.



Scheme 2. Experimental protocol. Black arrows indicate single steps of the experiment with blood-taking for the evaluation of acid–base balance. White arrows denote single steps of the experiment with measurement of the ventricular arrhythmia threshold for evaluation of the electrical stability of the heart.

periods to evaluate the acid-base balance in single experimental steps. Group 1 contained intact rats before surgical interventions, spontaneous breathing under ketamine/xylazine anaesthesia (Int). Group 2 contained rats after tracheotomy, thoracotomy and 5 min of normal artificial ventilation (Stabil). Group 3 contained rats who underwent 2 min of apnoea (Apnoea). Group 4 had rats that underwent 5 min of reoxygenation (5 reoxy). Group 5 had rats that underwent 10 min of reoxygenation (10 reoxy). Group 6 involved rats who had 15 min of reoxygenation (15 reoxy). Group 7 contained animals that had 20 min of reoxygenation (20 reoxy). The experiment was completed by taking blood samples (Scheme 2).

Stimulation protocol

The chest was opened via a parasternal thoracotomy for elimination of nervous breathing control mechanisms and measurement of the electrical stability of the heart (measured by the ventricular arrhythmia threshold (VAT)). The VAT was estimated as the minimal amount of electrical current (mA) needed for elicitation of ventricular arrhythmias. The heart was protected from changes in temperature and humidity by administering physiological solution, dropwise, to the heart, with temperature equal to rectal temperature measured before the anaesthetic agent application. The stimulating platinum electrodes (diameter 1 mm and 5 mm interelectrode distance) were placed at the base of right ventricle. Parameters of the electrical stimulation were 400 ms series of rectangular pulses; frequency, 30 Hz; impulse length, 10 ms). Stimuli were triggered by onset of the R wave in the II lead of electrocardiography (ECG) on the base of synchronization of ECG with stimulator. The current intensity was increased progressively by increments of 0.2 mA until ventricular arrhythmias were obtained. Recovery of the sinus rhythm was spontaneous. The time of experiment, from the application of the anaesthetic agent to the exit of animal, was 40 min.

Measurement of HR

Bipolar electrodes were attached to the upper and lower limbs and served for recording of the HR

and ECG, which was further analysed using computer software (ECG Practic Veterinary, Prague, Czech Republic). HR was measured (mean value of the last four cycles) in intact animals; after tracheotomy (Tr); thoracotomy (To); after each minute of the 5-min stabilization; after each 30 s of the apneic episode; and after each minute of the 20-min reoxygenation.

Statistical analyses

Data are mean ± SD. $p<0.05$ was considered significant by the non-parametric (Kruskal-Wallis) test. The relationship between evaluated parameters was determined by calculation of the correlation coefficient, where the interval $-0.4 > r > +0.4$ was considered significant. These data were averaged from trials that were undertaken independent of season, because circannual variation can also be present.

RESULTS

Electrical stability of the heart

The electrical stability of the heart after the period of stabilization showed significant ($p<0.001$) LD differences with the higher values during the active (dark) part of the regimen day (light, 1.88 ± 0.71 mA vs. dark 2.39 ± 0.89 mA). Apneic episodes significantly ($p<0.001$) decreased the VAT in both light parts of the day (light, from 1.88 ± 0.71 mA to 1.32 ± 0.44 mA; dark, from 2.39 ± 0.89 mA to 1.52 ± 0.85 mA) and eliminated LD differences. Although 5 min of reoxygenation significantly ($p<0.001$) increased the VAT (light, from 1.32 ± 0.44 mA to 2.03 ± 0.89 mA; dark, from 1.52 ± 0.85 to 2.22 ± 0.96 mA), it did not permit recovery of the LD differences.

LD differences were found after the 15th minute (light 1.86 ± 0.74 mA *vs.* dark 2.62 ± 0.89 mA) and after the 20th minute of reoxygenation (light 1.78 ± 0.54 mA *vs.* dark 2.69 ± 0.63 mA). In the dark part of the day, the VAT tended to increase with the duration of reoxygenation, whereas the opposite tendency was seen in the light part of the day (Figure 1).

Heart rate

The changes in HR in spontaneous-breathing animals, after Tr, after To, after each minute of 5-min stabilization as well as during apneic episodes were identical with the preservation of LD differences. A 30 s apneic episode significantly

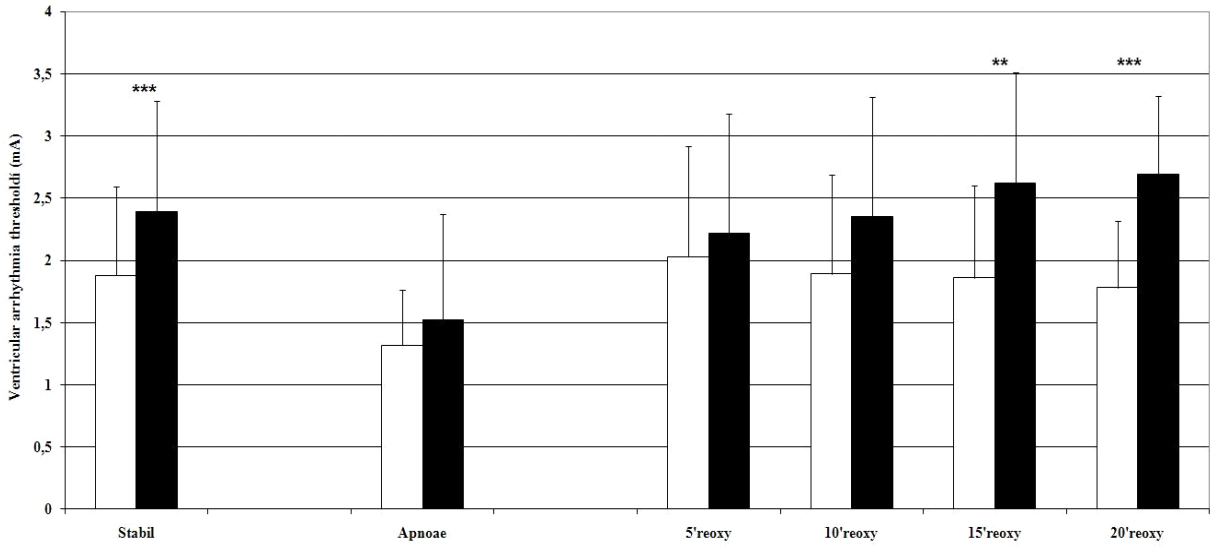


Figure 1. Average VAT±SD value changes in single experimental steps. White columns represent the light part of the day, and black columns the dark part. Stabil: animals after tracheotomy, toracotomy and 5 min artificial ventilation. Apnoea: after a 2-min apneic episode. 5 reoxy, 10 reoxy, 15 reoxy and 20 reoxy: after 5, 10, 15 and 20 min of reoxygenation, respectively. ***$p<0.001$; **$p<0.01$ statistically significant VAT differences between the light and dark part of the day.

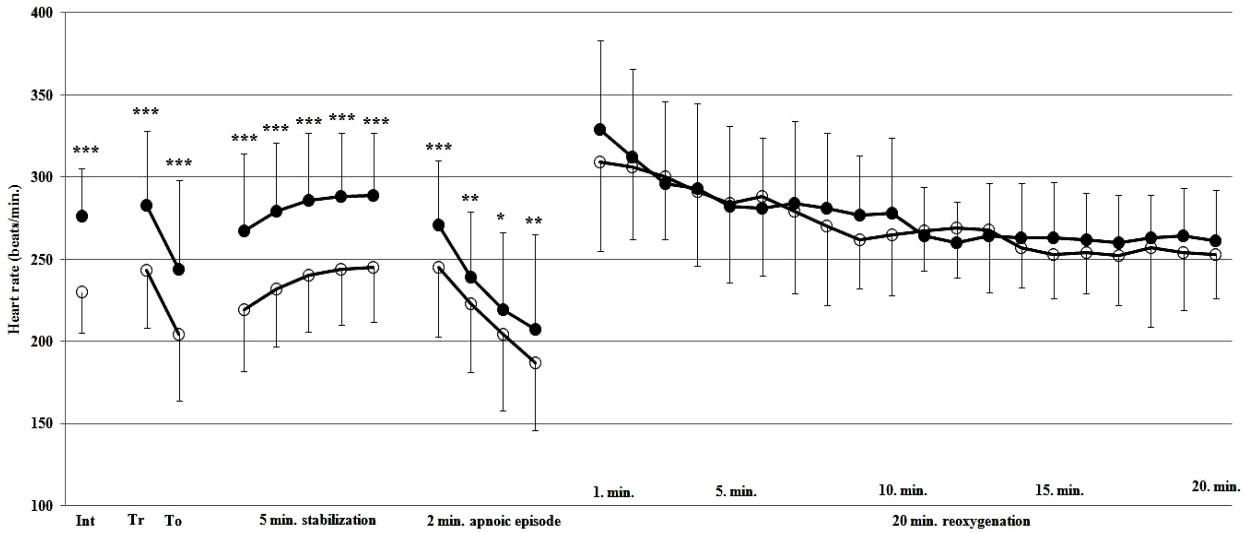


Figure 2. HR changes in single experimental steps. White circles represent the light part of the day and black circles the dark part. Int: intact animals under ketamine/xylazine anaesthesia before surgical intervention allowing for spontaneous breathing in the supine position. Tr: tracheotomy. To: thoracotomy. ***p<0.001; **p<0.01; *p<0.05 statistically significant HR differences between the light and dark part of the day.

(p<0.001) decreased the HR only in the dark part of the day compared with the HR value from the end of the period of stabilization (30 s of apnoea 271 ± 39 beats/min. *vs.* Stabil 289 ± 38 beats per minute (bpm)). No statistical significant decrease was found in the light part. The HR decreased gradually until 120 s of the apneic episode in both lighted parts of the day (light, 245 ± 42 bpm, 223 ± 42 bpm, 204 ± 46 bpm and 189 ± 41 bpm; dark, 271 ± 39 bpm, 239 ± 40 bpm, 219 ± 47 bpm and 189 ± 41 bpm). Although reoxygenation significantly (p<0.001) increased the HR in both lighted parts of the day (light, from 189 ± 41 bpm to 309 ± 54 bpm; dark, from 189 ± 41 bpm to 329 ± 54 bpm), LD differences were eliminated during the entire duration of reoxygenation (Figure 2). Twenty minutes of reoxygenation recovered the HR approximately to the HR levels of spontaneously breathing animals.

Calculation of the correlation coefficients between the HR and VAT did not reveal a relationship in the single experimental steps except the light part of the day after apneic episodes, in which a moderate negative correlation (r = –0.47) was found. It means that greater electrical stability was associated with a lower heart rate at the time.

DISCUSSION

The current study shows that the LD cycle significantly influences myocardial vulnerability to ventricular arrhythmias as well as HR at baseline and during reoxygenation in an anaesthetized rat model. By contrast, there was no impact of the LD cycle during apneic episode when such vulnerability was maximal.

The first limitation of the present study was a lack of data from conscious animals for a comparison of the effect of anaesthesia. This type of anaesthesia probably modifies the acrophase, mesor and amplitude of daily rhythms but without the loss of the daily rhythmicity [15, 16]. The preservation as well as elimination of LD dependence after surgical interventions in the experimental model did not confirm or exclude the hypothesis that surgery compared with the simple effect of the anaesthetic agent can have a more marked effect depending on the LD cycle [17]. The second limitation was that rats were in systemic hypoxia from the start to the end of the experiment independent of the LD cycle. This state has been described by other authors in rats [18, 19] and reflects the changes in cardiovascular [18] and respiratory [20] systems.

The disruptive effect of acute hypoxia on the LD-dependent differences in the HR–response curve was not confirmed, as suggested by other authors [21-25]. One of the main conclusions from the present study was that the HR was significantly and systematically higher in the dark part than in the light part of the day as well as during apneic episode-induced acute systemic asphyxia. Similarly, hypercapnic hypoxia may contribute to a larger parasympathetic influence on the heart [26], similar to the asphyxia used in the present work. Bradycardia can be the result of ketamine/ xylazine anaesthesia, which expressively increases parasympathetic and decreases sympathetic drive in rats (Svorc, Jr., unpublished observations). Although HR values were on the same level as that seen in bradycardia, serious acute asphyxia was regularly accompanied by HR decrease in both lighted parts of the day. Afferent inputs, behavioural states and sex-dependent differences may have an additional role. An increase in HR occured in males but not in females after exposure to intermittent hypoxia [27].

The results from our model clearly demonstrate and confirm the pro-arrhythmogenic effects of apneic episodes (considerable systemic asphyxia, bradycardia and a decrease in the electrical stability of the heart) [6-10, 28]. Our data demostrate that the pro-arrhythmogenic effects of apneic episodes act in the same manner regardless of whether animals are in the light (non-active) or dark (active) part of the day. However, it seems that recovery of the pulmonary ventilation can attenuate or amplified pro-arrhythmogenic effect of the apneic episode dependent on synchronization to the LD cycle. In the dark part of the day, the gradual increase in the VAT is associated with the duration of reoxygenation (anti-arrhythmogenic effect) compared with the light part of the day, where the contrary tendency was observed (pro-arrhythmogenic effect). This suggests that synchronisation of the organism to a particular external periodicity could be a crucial factor influencing myocardial vulnerability to ventricular arrhythmias mainly in the process of recovery of the pulmonary ventilation after an apneic episode.

CONCLUSIONS

The model suggests that synchronisation to local time may be an important factor for evaluation of cardiovascular risks in patients suffering from OSA. Analyses of myocardial reactions to acute systemic asphyxia induced by apneic episodes (as well as to reoxygenation) is very important in experimental respirology and cardiology because the myocardium reacts differently depending on the external environmental periodicity.

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