

Effect of Systemic Hypoxia and Reoxygenation on Electrical Stability of the Rat Myocardium: Chronophysiological Study

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Summary

The aim of the study was to determine the dependence of changes in the electrical stability of the heart on the light-dark cycle (LD cycle) in disorders of pulmonary ventilation. The ventricular arrhythmia threshold (VAT) was measured in female Wistar rats (adaptation to the light regime 12:12 h, ketamine/xylazine anesthesia 100 mg/15 mg/kg, i.m., open chest experiments). The conditions of the normal artificial ventilation and reoxygenation were $V_T = 1$ ml/100 g, respiratory rate 40 breaths/min, hypoventilation $V_T = 0.5$ ml/100 g, respiratory rate 20 breaths/min. The animals (n=11 light group; n=19 dark group) were subjected to 20 min hypoventilation followed by 20 min reoxygenation. The control prehypoventilatory VAT differences were not found between the light (1.90 ± 0.84 mA) and dark (1.88 ± 0.87 mA) part of the day. Artificial hypoventilation changed the VAT values in light and dark part of the day differently. While during the light period, the average VAT values in most animals (90.9 %) were significantly decreased (1.29 ± 0.59 vs. 1.90 ± 0.84 mA control, $p < 0.05$), during the dark part these values showed either significant increase (63.2 %) (2.23 ± 0.77 vs. 1.48 ± 0.39 mA, $p < 0.005$) or a slight non-significant decrease (36.8 %) (2.18 ± 0.89 vs. 2.54 ± 0.99 mA). Reoxygenation returned the VAT values to the level before hypoventilation by an increase of the VAT (81.8 %) in the light part of day and by decrease of the VAT (68.4 %) in the dark part of the day. It is concluded that 1) in hypoventilation/reoxygenation model, the significant higher average VAT values are in the dark part of the day vs. the light one, 2) rat hearts are more resistant to systemic hypoxia and reoxygenation in the dark part of day, and 3) proarrhythmic effect of the systemic hypoxia is only seen in the light part of the day.

Key words

Electrical stability of the heart • LD cycle • Hypoventilation • Rat

Introduction

Most physiological functions of the living organism, especially those of the cardiovascular system, show a marked circadian rhythmicity (Henry *et al.* 1990). The circadian fluctuations concern both blood pressure

and heart rate, but also the incidence of ventricular dysrhythmias, onset of cardiovascular symptoms and manifestations of cardiovascular diseases.

The onset and development of ventricular arrhythmias depends on many factors to which some disorders of pulmonary ventilation also belong. The

effect of systemic hypoxia, hypercapnia and acidosis (consequences of hypoventilation) were studied not only in animal experiments (Kujaník *et al.* 1984, 1985, Tomori *et al.* 1997, 2000, Švorc *et al.* 2003) but also in clinical trials (Guilleminault *et al.* 1983, Peter 1990, Kujaník *et al.* 2000a, 2000b). Surprisingly, there are few reports describing the daytime of the experiment running or synchronization of animals to the LD cycle. Papers, studying factors responsible for the onset and development of ventricular arrhythmias, have mainly focused on the temporally current mechanical and metabolic changes in the myocardial cells, often irrespective of the circadian dependence. This can be a problem, because the LD cycle is one of the strongest circadian synchronizers of the animal endogenous rhythms. For this reason, the circadian variability should be considered as an important factor especially in the cardiovascular studies.

In animal experiments, the link between disorders of pulmonary ventilation and incidence of ventricular arrhythmias was also demonstrated in circadian dependence (Otsuka and Watanabe 1990, Švorc *et al.* 1997, 2000a). Using the 24 h chronogram Otsuka and Watanabe (1990) found that the hourly distribution of the apnoe index coincided with the highest incidence of bradyarrhythmias in rats. In the hypoventilation-reoxygenation rat model under pentobarbital anesthesia, hypoventilation decreased the VAT and circadian rhythm acquired a moderate biphasic character (Švorc *et al.* 1997). Reoxygenation changed the VAT circadian rhythm to inverse against a monophasic one during normal ventilation (Švorc *et al.* 2000a). It means that the LD cycle can play a role in the development of hypoxia/reoxygenation induced ventricular arrhythmias and can belong to the group of predisposing factors.

The question remains whether vulnerability of the ventricles to arrhythmias is primarily changed only by the factors resulting from the changed ventilation, or if there are factors, as anesthesia, or also natural factors (LD cycle), which can influence the followed parameter. The aim of our study was to evaluate the effect of the LD cycle on the electrical stability of the rat heart under ketamine/xylazine anesthesia under the conditions of the changed ventilation.

Methods

Experimental animals and conditions of adaptation

The experimental procedures were performed in

accordance with the Helsinki Declaration for scientific experimentation on animals. The experiments were performed on anesthetized (ketamine/xylazine anesthesia, ketamine 100 mg/kg Narkamon SPOFA Prague + xylazine 15 mg/kg Rometar SPOFA Prague, i.m.) female Wistar rats (3-4 months old). The rats were adapted to a light and climate-controlled room (relative moisture from 40 % to 60 %, temperature 24 °C in the cages) for 4 weeks. The rats were kept in the cages (3 animals/cage) and had free access to food and water.

Experimental groups and protocol

The effect of the light period (n=11; light group) was followed after adaptation to LD cycle of 12:12 h, with the dark part of day from 18:00 to 06:00 h. The VAT measurements were performed twice (the first animal between 09:00-10:00 and the second one between 12:00-13:00 h). The effect of the dark period (n=19, dark group) was followed after inverse setting of LD cycle, with the dark period from 06:00 to 18:00 h, with the times of measurement as in the first case (see scheme in Fig. 1). The experiments were performed during the whole year and the results were averaged independently of the season.

The heating of animals was performed before the surgical interventions (tracheotomy and thoracotomy) to the value of the rectal temperature measured before the anesthetic application. After tracheotomy and thoracotomy, animals were ventilated for 5 min at normal ventilation (see "Conditions of ventilation") and then they were subjected to 20-min hypoventilation followed by 20-min reoxygenation.

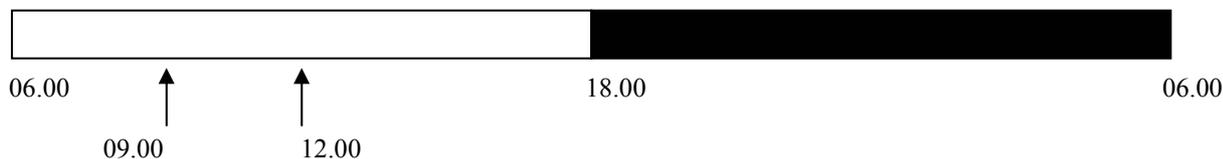
Conditions of ventilation

The pulmonary ventilation was sustained through a tracheal cannula connected to an artificial respirator. The parameters of normal ventilation and reoxygenation were as follows: respiratory rate 40 breaths/min, tidal volume 1 ml/100 g of body weight. The respiratory rate for hypoventilation was 20 breaths/min, tidal volume 0.5 ml/100 g of body weight. The analysis of the blood gases and acid-base balance by ASTRUP method was used for the monitoring of the respiratory effect of ventilation. The changes in the blood gases and acid-base balance were detected from blood samples taken from the femoral artery after 5 min stabilization (pH_a 7.44±0.11, paO_2 10.8±1.7 kPa, $paCO_2$ 3.22±1.3 kPa, O_2 saturation 91.6±9.9 %), at the end of 20-min hypoventilation (pH_a 7.15±0.07, paO_2 7.3±1.7

kPa and paCO_2 7.3 ± 1.3 kPa, O_2 saturation 68.2 ± 13.7 % and 20-min reoxygenation (pH_a 7.37 ± 0.06 , paO_2 9.46 ± 1.85 kPa and paCO_2 4.62 ± 0.71 kPa, O_2 saturation 93.3 ± 3.6 %), respectively. Whereas the average values

paO_2 , paCO_2 a pH_a between light and dark part of the day were practically identical, the above mentioned average values are common for both light parts.

The light phase of experiment (light group)



The dark phase of experiment (dark group)

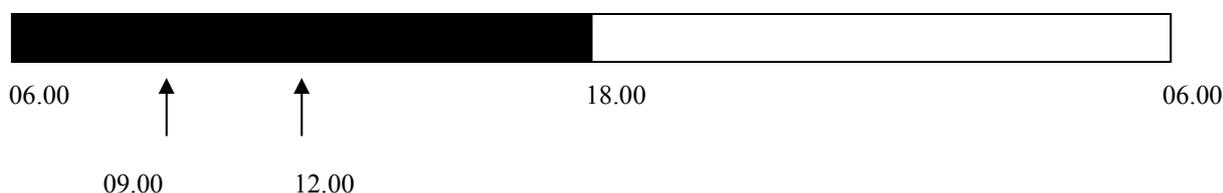


Fig. 1. Scheme of adaptation of the animals on LD cycle 12: 12 hours. Empty bar - light part of rat regime day (in the light group from 06.00 to 18.00 o'clock, in the dark group from 18.00 to 06.00 o'clock), full bar - dark part of rat regime day (in the light group from 18.00 to 06.00 o'clock, in the dark group from 06.00 to 18.00 o'clock). Arrows - the time of experiments.

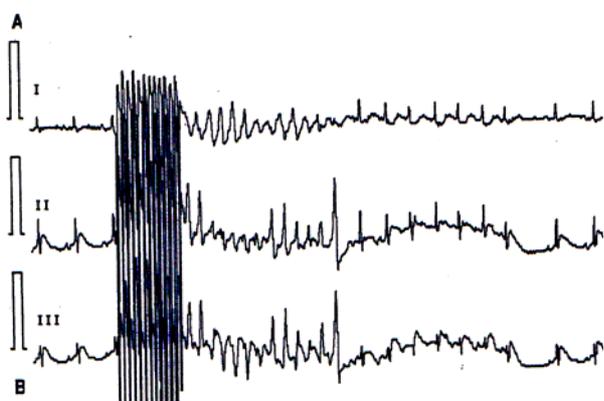


Fig. 2. Representative ECG records (limb leads I, II, III) of ventricular arrhythmia induced by the train of electrical stimulation of right ventricle.

Measurement of VAT

The control VAT values were measured after the surgical interventions (tracheotomy and thoracotomy) and a 5-min period of stabilization with the parameters of the normal pulmonary ventilation. The effect of hypoventilation and reoxygenation on the VAT was followed after 5, 10, 15 and 20 min of ventilation. The VAT was measured directly by electrical stimulation of the heart. The stimulating electrodes (diameter 1 mm and

5 mm distance between electrodes) were fixed at the base of the right ventricle in the supine position. Cardiac stimulation (rectangular pulses with a frequency 30 Hz, 10 ms impulse length, and duration of stimulation 400 ms) was triggered by the initial pulse of the R wave. The current intensity was increased progressively by steps of 0.2 mA until ventricular arrhythmias were obtained. The onset and course of the ventricular arrhythmias was evaluated visually by monitoring of ECG from bipolar limb leads using computer system ECG Practic Veterinary (Fig. 2). Resuscitation was spontaneous, 4-5 min after termination of the ventricular arrhythmias.

Statistical analysis

The data are presented as means \pm S.D. Statistical levels $p < 0.05$ were considered to be significant by non-parametric tests. The effect of LD cycle on the VAT changes was tested by the χ^2 -test.

Results

No significant differences in control VAT values (measured during normal artificial ventilation for 5 min) were found between the light and dark part of the day

(1.90 ± 0.84 mA light vs. 1.88 ± 0.87 mA dark) (Fig. 3).

Artificial hypoventilation changed the VAT values differently in the light and dark part of the day. While during the light part, the average VAT value was significantly decreased (1.29 ± 0.59 mA hypo vs. 1.90 ± 0.84 mA control, $p < 0.05$), during the dark part the

VAT value showed non-significant increase (Fig. 3). The data collected in intervals through the hypoventilation (20 min) showed that the VAT values were significantly higher in dark part of day as compared to the light part of the day (Fig. 4).

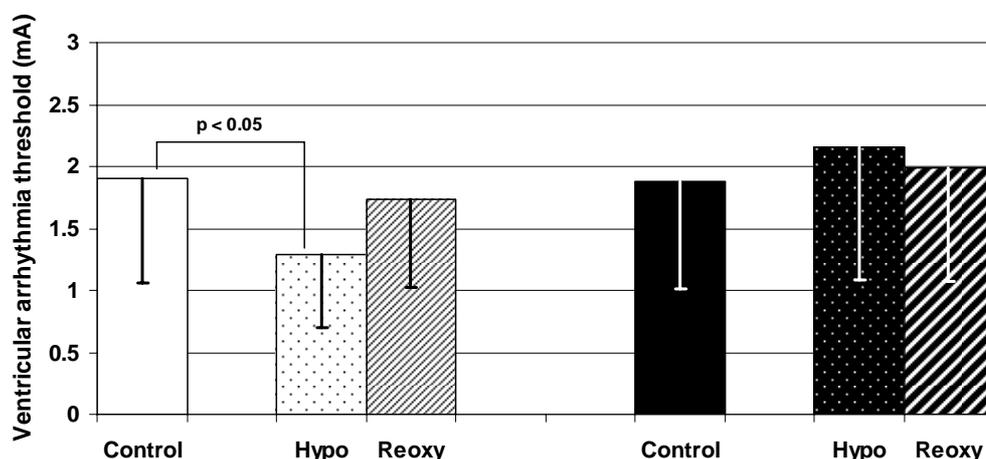


Fig. 3. The changes of the VAT in the hypoventilation/reoxygenation model in the light and dark part of day. Control - VAT value after the surgical interventions (tracheotomy, thoracotomy) and 5 min stabilization at the parameters of normal artificial pulmonary ventilation (empty column - light group, full column - dark group). Hypo - the average VAT value from measurements after 5, 10, 15 and 20 min of hypoventilation (light dotted columns - light group, dark dotted column - dark group). Reoxy - the average VAT value from measurements after 5, 10, 15 and 20 min of reoxygenation (light hatched column - light group, dark hatched column - dark group).

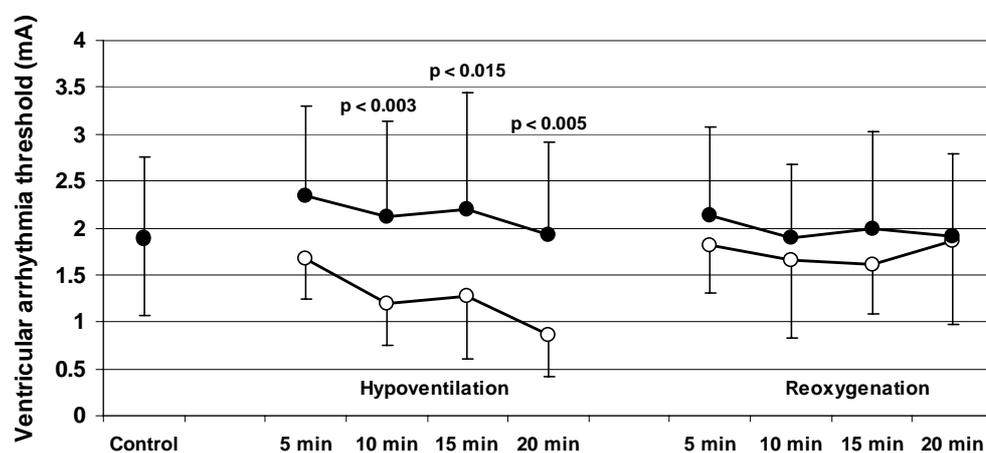


Fig. 4. Changes of the VAT in the individual intervals of measurement. The values are presented as arithmetical means \pm SD (open circles - the light part of day; full circles - the dark part of day). Statistical significance refers to differences between the light and dark part of day.

Reoxygenation returned the VAT values to the level prior to hypoventilation in a different manner during the light and dark part of the day (Fig. 3). Recovery was reached by an increase of VAT in the light part of the day (1.74 ± 0.72 mA reoxy vs. 1.29 ± 0.59 mA hypo vs.

1.90 ± 0.84 mA control) and by the decrease of VAT in the dark (1.99 ± 0.92 mA reoxy vs. 2.16 ± 1.07 mA hypo vs. 1.88 ± 0.87 mA control). In the dark part of day, reoxygenation further caused profound alterations in the VAT gained by hypoventilatory hypoxia (Fig. 4).

Although VAT was attained by a different route, the average VAT values were not significantly different between light and dark part of the day.

Hypoventilation as well as recovery of ventilation did not produce the same myocardial response to electrical stimulation in individual animals. The reactions of animals to electrical stimulation under different ventilation in the both light parts of day are

shown in Table 1. χ^2 -test was performed on the basis of these individual responses in the aspect of previous threshold values for evaluating the effect on the LD cycle. The significant effect of the LD cycle on the VAT changes was confirmed for prolonged periods of hypoventilation ($p < 0.05$) as well as reoxygenation ($p < 0.01$), respectively.

Table 1. Individual responses of animals to electrical stimulation. Numerator - number of animals with VAT changes against the previous VAT measurement, denominator - number of animals in experimental group.

	Hypoventilation vs. control value		Reoxygenation vs. hypoventilation	
	Light	Dark	Light	Dark
VAT decrease	10/11 (90.9 %)	7/19 (36.8 %)	2/11 (18.2 %)	13/19 (68.4 %)
VAT increase	1/11 (9.1 %)	12/19 (63.2 %)	9/11 (81.8 %)	6/19 (31.6 %)

Discussion

The individual VAT values, measured during experiments, showed wide inter- as well as intraindividual variability. The explanation of such a variability can be due to production of spontaneous unpredictable alterations in the electrical stability of the heart induced by anesthesia or hormonal and homeostatic reflexes operating only in intact animals (Lubbe *et al.* 1975). It can be a limitation of this study that there is no uniform and predictive reaction of animals to anesthesia or to changed ventilation.

The electrical stability of the myocardium is mainly influenced by changes of ion distribution. Although the effect of the LD cycle on ion concentrations was not the aim of our work, the studies of other authors (Stoynev *et al.* 1986, Poulis *et al.* 1989, Lausson *et al.* 1985, Roelfsema 1987) refer to circadian variability of ion concentrations under normal ventilatory conditions. Thus LD changes in the electrical stability of the heart might reflect the LD changes in ion concentrations. The mean values of control VAT measurements, however, show that there are no differences between the light and dark part of the day. Loss of VAT dependence on the LD cycle refer to the fact that ketamine/xylazine anesthesia probably minimizes or disturbs the effect of the LD cycle on rat myocardial vulnerability under the conditions of the normal pulmonary ventilation. Our results are consistent with results of Bruguerolle's group, who demonstrated the perturbations of daily rhythm of heart rate, locomotor activity and body temperature in rats

under ketamine anesthesia (Prudian *et al.* 1997). Almost 10 days were needed to detect a significant daily rhythm for above mentioned parameters (Pelissier *et al.* 1998).

Systemic hypoxia induced by hypoventilation changed the electrical stability of the rat heart in dependence on the LD cycle. Although the VAT decreased concomitantly in both light parts of the day during 20-min hypoventilation, it was demonstrated that: 1) the significantly higher average VAT values were in the dark part of day (active phase) against the light one (sleep phase), 2) the rat hearts are more resistant against the systemic hypoxia in the dark part of day, and 3) the significant decrease of the VAT refers to the proarrhythmogenic effect of the systemic hypoxia only in the light part of day. These differences probably result from the changed myocardial reactivity to electrical stimulation in dependence on the LD cycle. An important and still open question remains, whether the mechanisms responsible for the changed myocardial vulnerability are mobilized mainly by hypoventilation-induced systemic hypoxia, hypercapnia and acidosis with the additive effect of the LD cycle, or whether they are mobilized by the factors oscillating in the circadian dependence and with an additive effect of hypoventilation. This question is important because some reports describe disruptive effect of hypoxia on circadian rhythms, for example, on body temperature and locomotor activity in rats (Bishop *et al.* 2000, 2001, Mortola and Seifert 2000) and in golden hamsters (Jarsky and Stephenson 2000). Hypoventilation induced systemic hypoxia has non-unequivocal influence on changes of the P, R and T

amplitudes of ECG (Štimmelová et al. 2004) but significantly influences the PQ, QT and QTc durations in dependence on the light and dark phase of the rat regime day (Štimmelová et al. 2002).

Although reoxygenation returned the VAT to control values in both light and dark part of the day, the problem remains that the VAT was increased compared to hypoventilatory value only in the light part of day. A contrary tendency was found in the dark part of the day. The decrease in the dark part of day can probably signalizes the larger extent of reoxygenation injury of the myocardium in the dark (active) part of day. This fact is supported by our previous results in the rats under pentobarbital anesthesia. During reoxygenation after hypoventilation, the highest myocardial vulnerability was found between 24:00 and 3:00 h (after adaptation on LD cycle 12:12 h, dark part of the day from 18:00 to 6:00 h), but at the normal artificial pulmonary ventilation, the minimal vulnerability was exactly the same during this period (Švorc et al. 2000b).

It is concluded that although the electrical

stability of the rat heart under ketamine/xylazine anesthesia does not show a dependence on the LD cycle during normal pulmonary ventilation (probably due to the effect of ketamine/xylazine anesthesia), hypoventilation and reoxygenation change myocardial vulnerability in the dependence on LD cycle. It seems that rat myocardium is probably more sensitive to hypoventilation induced by systemic hypoxia mainly in the light (non-active) part of day. The conclusions of this study show that the time of the experiment or synchronization to the LD cycle and the circadian rhythmicity in the rat myocardial sensitivity to hypoxia should be considered as important factors, which can influence the results in experimental cardiology. Information about the circadian changes of myocardial sensitivity to hypoxia extend our knowledge about the mechanisms, which are responsible for the effect of systemic hypoxia on the myocardium.

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Reprint requests

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