EXPERIMENTAL STUDY

Chronophysiologic aspects of ECG changes during a systemic asphyxic episode and subsequent reoxygenation in an experimental rat model

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Abstract: The aim of the study was to evaluate the effect of ventilatory manoeuvres on some ECG parameters as a function of the light-dark (LD) cycle in in vivo conditions. The PQ and QT intervals were measured in ketamine/xylazine-anaesthetized female Wistar rats (100 mg/15 mg/kg, i.m.) after adaptation to an LD cycle (12:12 h). The animals were exposed to a 2 min apneic episode and subsequent 20 min period of reoxygenation. Significant LD differences were found in the duration of the PQ interval (p<0.001) after 30 and 60 sec., and in the QT interval after 90 (p<0.01) and 120 sec. (p<0.001), apneic episode. Reoxygenation restored the PQ and QT intervals with the preservation of LD differences from the pre-asphyxic period. It is concluded that although long-term asphyxia probably minimized LD differences in the duration of the PQ interval, the dispersion of refractory periods increases by the manner depending on LD cycle. Reoxygenation did not act proarrhythmogenically and the followed parameters were recovered to the pre-asphyxic level (Fig. 5, Ref. 43). Full Text (Free, PDF) www.bmj.sk.

Key words: hypoxia, ECG changes, LD dependence, myocardium, rat.

Most physiologic functions, especially those involving the cardiovascular system, show a marked circadian rhythmicity (Henry et al, 1990). The fluctuations in the dependence on daytime concern blood pressure and heart rate (Waterhouse et al 2000, Zhang and Sannajust, 2000), electrophysiologic properties of the heart (Švorc et al, 2005), the incidence of ventricular dysrhythmias (Fries et al, 2001, Taneda and Aizawa, 2001) as well as manifestations of cardiovascular diseases (Steinbigler et al, 1999).

The onset and development of ventricular arrhythmias depend on many factors, to which some disorders of pulmonary ventilation belong. The significant effects of systemic hypoxia, hypercapnia, and acidosis on the development of various ventricular arrhythmias have been reported in experimental animal studies (Kujanik et al, 1984, Tomori et al, 1997, 2000) and clinical trials (Guillemainault et al, 1983, Peter, 1990, Kujanik et al, 2000a). Acute systemic asphyxia causes profound electrophysiologic disorders of the myocardium in the conductive system (Dhalwal, 1991, Kujanik, 2000b, Stimmelova et al, 2002, 2004), the distribution of refractory periods (Stimmelova et al, 2004) and changes of the heart rate (Graf, 2006, Overgard et al, 2007), as well as of the threshold for ventricular arrhythmias (Švorc et al, 2000). All these changes lead to various dysrhythmias and finally to myocardial infarction (Motte et al, 1994).

The chronobiologic aspects of the connection between decreased pulmonary ventilation (hypoventilation) or cessation of ventilation (apneic episode) and the changes of the electrophysiologic myocardial properties are less well-documented. Although this dependence has been demonstrated in experimental animal models (Bishop et al, 2000, Jarsky and Stephenson, 2000, Švorc et al, 2000), many studies involving the factors responsible for the onset and development of ventricular arrhythmias have focused mainly on the temporally current mechanical and metabolic changes in myocardial cells, often irrespective of the circadian dependence. It is known that cardiac function shows a marked circadian rhythmicity and the light-dark cycle is the strongest synchronizer of endogenous animal rhythms. There are only sparse reports describing the daytime dependence of experiments using the synchronization of animals to the light-dark cycle. However, whether the vulnerability of the myocardium to ventricular arrhythmias is influenced purely by ventilation-induced systemic asphyxia or by additional factors, such as anaesthesia, or natural factors, such as changes in the light-dark cycle, remains to be determined.

Electrophysiologic properties or an electrophysiologic predisposition to heart rhythm disorders can be determined directly from the ECG record. Thus, the aim of the present study was to evaluate the effect of ventilatory apneic episode-induced systemic asphyxia and restoration of ventilation on changes in some

Indexed and abstracted in Science Citation Index Expanded and in Journal Citation Reports/Science Edition
ECG parameters, referring to the possible cause of the onset or development of heart rhythm disorders in the anaesthetized rat model as a function of the light-dark cycle.

**Methods**

**Experimental animals and conditions of adaptation**

The experiments were performed in 3–4 month old female Wistar rats with ketamine/xylazine anaesthesia (ketamine [Narcofan], 100 mg/kg; SPOFA, Prague, Czech Republic, xylazine (Rometar), 15 mg/kg; SPOFA Prague, Czech Republic, l.m.) after 4 weeks of adaptation to the light-dark cycle (12:12 h) in a controlled room with relative moisture (40 %) and temperature (24 °C) in the cages. The rats were kept in cages (2 animals/cage) and had access to food and water *ad libitum*. The studies conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996). Upon completion of the experiments, the animals were euthanized by the cardiac administration of a ketamine overdose.

The effect of the light period (the first phase) was followed after adaptation to the LD cycle (12h L : 12h D) for 4 weeks, with the dark part of day from 18.00 to 06.00h. The experiments were performed twice in the course of the period (the first animal between 09.00 and 10.00h, and the second animal between 12.00 and 13.00h). The effect of the dark period (the second phase) was followed after an inverse setting of the LD cycle, with the dark part of the day from 06.00–18.00h, with the times of recording as in the first case (Fig. 1).

**Ventilation techniques**

The animals were in the supine position on a pre-heated table, and the trachea was exposed at the midcervical level and cannulated by a plastic tube. The tracheal cannula was attached to a volume-rate-regulated artificial ventilator (UGO BASILE, Como-Rio-Varese, Italy) and the animals were ventilated with humidified room air. The parameters of the initial ventilation and reoxygenation were as follows: respiratory rate, 40 breaths/min; and tidal volume, 1 ml/100 g of body weight. Apneic episodes were simulated by switching off the respirator for 2 minutes.

**Measurement of ECG**

The chest was opened by a parasternal thoracotomy, and after gentle mediastinal preparation, the heart was exposed for measurement of the electrical stability of the heart. Bipolar electrodes were attached to the upper and lower limbs for purposes of ECG recording, which was further analysed using a computer system (ECG Practic Veterinary, Prague, Czech Republic). The ECG was recorded in intact animals before surgical interventions in the supine position at spontaneous breathing (light, n= 116; dark, n=134), after tracheotomy (light, n=116; dark, n= 112), after thoracotomy (light, n=116; dark, n=110), after 5 min of stabilization with normal artificial ventilation without ventilatory manoeuvres (light, n=116; dark, n=110), after each 30 sec. apneic episode (light, n=94; dark, n=118), and after 5, 10, 15, and 20 min of artificial reoxygenation (light, n=30; dark, n= 50).

**Statistical analysis**

The data are presented as mean±SD. A non-paired Student’s t-test was used for statistical evaluation. Differences of p<0.05 were considered significant. The experiments were performed during the entire year and the results were averaged independent of the seasons.

**Results**

Significant LD differences in the duration of the PQ interval were observed in ketamine/xylazine anaesthetized intact animals (light, 47.7±11.2 ms vs dark, 36.5±5.8 ms). The surgical interventions, as well as the period of stabilization, did not disturb the LD dependence on the duration of the PQ interval with a significantly shorter duration (p<0.001) in the dark part of the day in contrast to the light part of the day (Fig. 2). Apneic episodes prolonged the duration of the PQ interval with significant preservation of the LD differences after 30 sec. (light, 47.7±11.0 ms vs dark, 37.5±5.8 ms; p<0.001) and 60 sec. (light, 51.3±11.7 ms vs dark, 46.7±9.1 ms, p<0.01). Apneic episodes of 90 sec. and 120
sec. abolished the LD differences. Reoxygenation shortened the duration of the PQ interval and restored significant LD differences (p<0.001) (Fig. 3).

No significant LD differences in the duration of the QT interval were found in intact animals and after surgical interventions (Fig. 4). Significant LD differences existed after 90 sec. (light, 102.6±13.3 ms vs dark, 108.8±17.3 ms; p<0.01) and 120 sec. (light, 98.1±18.6 ms vs dark, 108.8 ±16.4 ms; p<0.001) of apneic episodes with a longer duration in the dark part of the day. Significant LD differences (p<0.05) in the QT interval duration were found after 15 min (light, 90.1±11.2 ms vs dark, 95.2±9.5 ms) and 20 min (light, 88.4±9.5 ms vs dark, 94.4±61 ms) of reoxygenation (Fig. 5).

Discussion

A number of electrophysiological properties of the cardiac structures are recognized as essential for the triggering and maintenance of heart rhythm disorders and show dependence on the time of day (review Portaluppi and Hermida, 2007). The onset and development of heart rhythm disorders depends on many factors, to which some disorders of pulmonary ventilation (Fichter et al., 2002, Mehra et al., 2006) or cessation of ventilation (apneic episodes) (Arias and Sanchez, 2007, Patel and Rosen, 2007, Daccarett et al., 2008) also belong. For this reason, determination of the circadian link between disorders of pulmonary ventilation and changes in the electrophysiologic properties of the heart are important. The main aim of this study was to gain information concerning the chronophysiologic aspects of ECG time interval changes predisposing to the heart rhythm disorders after the ventilatory maneuvers – apneic episode-induced asphyxia and subsequent reoxygenation – in an in vivo rat model.

The values of the ECG parameters showed wide inter- and intra-individual variability in all groups. Such considerable variability of the results is a problem, mainly concerning in vivo studies, what was confirmed in our experimental group. It can be explained by production of spontaneous unpredictable alterations in the electrophysiologic properties of the heart induced by anaesthesia or hormonal and homeostatic reflexes in intact animals (Lubbe et al., 1975).

Our experimental results are in contrast as well as in agreement with other studies. The earlier studies of Prudian et al. (1997) and Pelissier et al. (1998) referred to the fact that ketamine anaesthesia perturbs the daily rhythm of the rat heart rate and the loss of heart rate rhythmicity can be also observed after surgical intervention as a reaction to the surgical stress. Our results did not affirm these conclusions because there were significant LD differences in the time of impulse transmission from the atria to the ventricles (PQ interval). Ketamine/xylazine anaesthesia, together with the initial systemic asphyxia (Mortola 2007) (decrease of pulmonary ventilation after the application of the anaesthetic agent), as well as surgical interventions, probably did not have any influence on the LD differences in the followed
parameter (see review by Portaluppi and Hermida, 2007). Controversially, the duration of the QT interval did not show a significant LD dependence. Similarly, the loss of diurnal variation of the dispersion of the refractory periods was also described in patients having either an ischemic or non-ischemic origin of heart failure treated with optimal drug therapy (Gunes et al, 2008).

The identical prolongations of the PQ and QT intervals affirm the effect of the apneic episode on the heart rhythm. Apneic episodes, obstructive or central often in a connection with the sleep apnea syndrome, are linked with disorders of the cardiac rhythm (Cutler et al, 2002, Bounhoure et al, 2005, Dunai et al, 2006, Bayram and Diker, 2008). From the clinical study, the significantly increased QT dispersion was also noted in the group of patients with myocardial infarction during sleep apneic episodes (Yamashita et al, 2004). LD differences are probably depressed by apnea-induced serious systemic asphyxia, which is in accordance with the opinion of the above mentioned authors about the effect of hypoxia on daily rhythm.

Similarly, there are contradictory conclusions about the effect of reoxygenation on the changes of the electrophysiological myocardial properties. Our results confirm the opinion of some authors (Perchenet and Krehrer, 1995, Bugge et al, 1997) about the anti-arrhythmogenic effect of reoxygenation. On the other side, there are papers, which describe a serious injury of the heart by the reoxygenation (Griffiths et al, 2000, Mukai et al, 2000). Despite various opinions and conclusions about the effect of asphyxia and recovery of pulmonary ventilation on the electrophysiological myocardial properties, the importance of our results are the designation that the myocardial vulnerability to the development of heart rhythm disorders depends on the LD cycle, not only in the intact anesthetized animals, but also after the ventilatory maneuvers. The short-term asphyxia does not disturb the LD dependence of the PQ interval and rat myocardium is more vulnerable for heart rhythm disorders originating from disorders of the impulse production and conduction in the light part vs. the dark part of the day. Although long-term asphyxia probably minimizes LD differences in the duration of the PQ interval, the dispersion of refractory periods increases by the manner depending on LD cycle and on the duration of asphyxia. Reoxygenation does not act proarrhythmogenically and the followed parameters recovered to the pre-asphyxic level in the ketamine/xylazine anesthetized rat model.

References


Received August 20, 2009. Accepted January 5, 2010.