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MYOCARDIAL ELECTROPHYSIOLOGY AND THE ACIDE-BASE BALANCE IN THE RAT CHRONOBIOLOGICAL STUDIES USING PENTOBARBITAL ANAESTHESIA

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ABSTRACT

General anaesthesia appears to disrupt cardiovascular stability by causing changes in cardiac function. The assessement of the chronobiological aspects of the pentobarbital anaesthesia impact on myocardial electrophysiology, acid-base balance and ion concentrations in a rat model was the aim of present study. The study was performed using pentobarbital-anaesthetized (40 mg/kg, intraperitoneal) female Wistar rats after adaptation to a 12h light : 12h dark (LD) cycle. Heart rate, rectal temperature, PQ interval, QT interval, QRS complex, QTc interval, and P, R and T wave amplitudes, acid-base balance and arterial concentrations of Na^+, K^+, Ca^{2+} and Cl^- were evaluated for their dependence on the LD cycle. LD differences were found in heart rate and rectal temperature measured before administration of the anaesthetic agent. Pentobarbital anaesthesia, however, eliminated LD differences in all electrophysiological parameters, parameters of acid-base balance and ion concentrations. LD differences with borderline statistical significance were found only for Na^+ levels, with a higher level in the light (ie, nonactive) period. Results of the present study suggest that pentobarbital is probably not the most suitable anaesthetic agent for chronobiological myocardial electrophysiological studies in rat models.

Key words: chronobiology, myocardial electrophysiology, acid-base balance, ion concentration; pentobarbital

INTRODUCTION

The use of general anaesthesia is usually necessary for ethically acceptable myocardial research involving animals. However, the agents used for general anaesthesia exert a variety of effects on the myocardium that may have significant impact on the incidence of arrhythmias (1). Thus, general anaesthesia appears to disrupt cardiovascular stability by causing changes in cardiac function, vascular reactivity and cardiovascular reflexes, and significantly alters the distribution of cardiac output to various organs (2).

The most commonly used anaesthetic agent in cardiovascular research is pentobarbital. Pentobarbital possesses cardiodepressive properties (3,4), induces increased reflex pressoreceptor activity (5) and has an antiarrhythmic effect (6,7). Pentobarbital anaesthesia modestly decreases blood pressure by reduction of the low-frequency component (sympathetic effect) of blood pressure variability (8) and thus appears to be closely related to the autonomic balance (9). Rats anaesthetized with pentobarbital exhibited a lower stroke index than ketamine-anaesthetized rats (10).

Dispersyn et al. (11) summarize the results from more chronobiological studies investigating interactions between general anaesthesia and circadian rhythms and concluded that general anaesthetics have a significant effect, which probably depends on the animal species. For example, pentobarbital induced both advanced and delayed phase shifts in the circadian rhythm of locomotor activity in SK mice; however, no phase shifts were observed at any circadian time with pentobarbital in C57BL mice (12). Furthermore, pentobarbital-induced phase shifts were not due to increasing activity levels (13). Pentobarbital had no apparent effect on the release of melatonin (14,15), on plasma atrial and brain natriuretic peptide levels (16) or locomotor activity in rats (15). Although pentobarbital anaesthesia has a cardiodepressive effect, it is not affect circadian fluctuations in rats.

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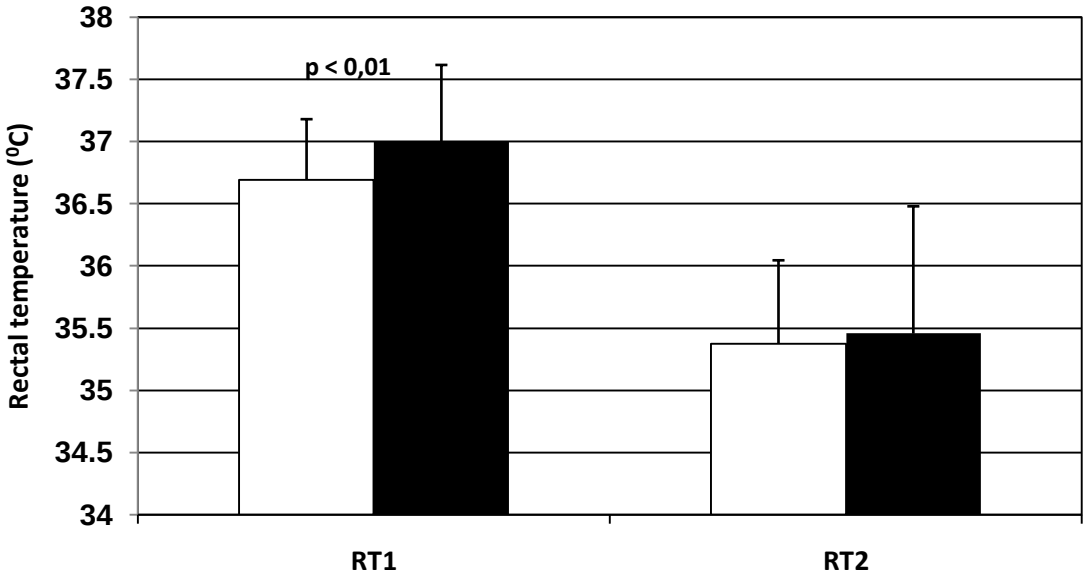

Figure 1 : Rectal temperature before (RT1) and after (RT2) administration of pentobarbital anaesthesia during the light (open bar) and dark (black bar) parts of the rat regimen day. Data presented as mean ± SD. p<0,01 was considered to be a statistically significant difference

With respect to dependence on the light-dark (LD) cycle under in vivo conditions in spontaneously breathing pentobarbital-anaesthetized rats, the specific aims of the present study were: to assess the light-dark changes in the rectal temeprature and in the heart rate, to assess electrophysiological myocardial parameters predicting the onset or development of heart rhythm disorders; and to assess the state of acid-base balance and selected ion concentrations in the arterial blood which have direct impact on the heart electrophysiology.

MATERIAL AND METHODS

Ethics approval, adaptation and anaesthesia

The present study conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (NIH publication number 85-23, revised 1996). The study protocol was also approved by the Ethics Committee of the Medical Faculty of Safarik University (Kosice, Slovak Republic) (permission number 2A/2015).

The present study was performed using two independent groups of female Wistar rats (mean [± SD] weight 310±20 g; three to four months of age) after adaptation to an LD cycle (12h light:12h dark; 40% to 60% humidity; cage temperature 24°C; two animals/cage; ad libitum access to food and water) for four weeks. In the first group (light group [n=16]), the effect of the light period on the monitored parameters was examined after adaptation to an LD cycle, with the light period from 06:00 h to 18:00 h. In the second group (dark group [n=27]), the effect of the dark period was monitored after adaptation to the inverse setting of the LD cycle, with a light period from 18:00 h to 06:00 h. The experiments were performed once in each animal in the course of a single LD period (the first animal between 09:00 h and 10:00 h and the second between 12:00 h and 13:00 h). The animals were anaesthetized (40 mg/kg intraperitoneal) using pentobarbital (SPOFA Prague, Czech Republic). All parameters were measured while the animals were under the influence of pentobarbital anaesthesia, except rectal temperature, which was measured before anaesthetic agent administration. On completion of the experiments, the animals were euthanized by an overdose of pentobarbital injected directly into the heart.

Measurement of rectal temperature

Rectal temperature was measured using a quick-run thermometer immediately before administration of pentobarbital (RT1) according to standard protocol of the animal handling. The second value of the rectal temperature (RT2) was measured in the animals supine position, approximately 15 min. after pentobarbital administration.

Electrophysiological parameters

Heart rate and electrocardiographic (ECG) parameters (PQ interval, QT interval, QRS complex, QTc interval, and P, R and T wave amplitudes) were measured to the animals in supine position and during spontaneous breathing on a preheated table, and analyzed using the II bipolar limb lead

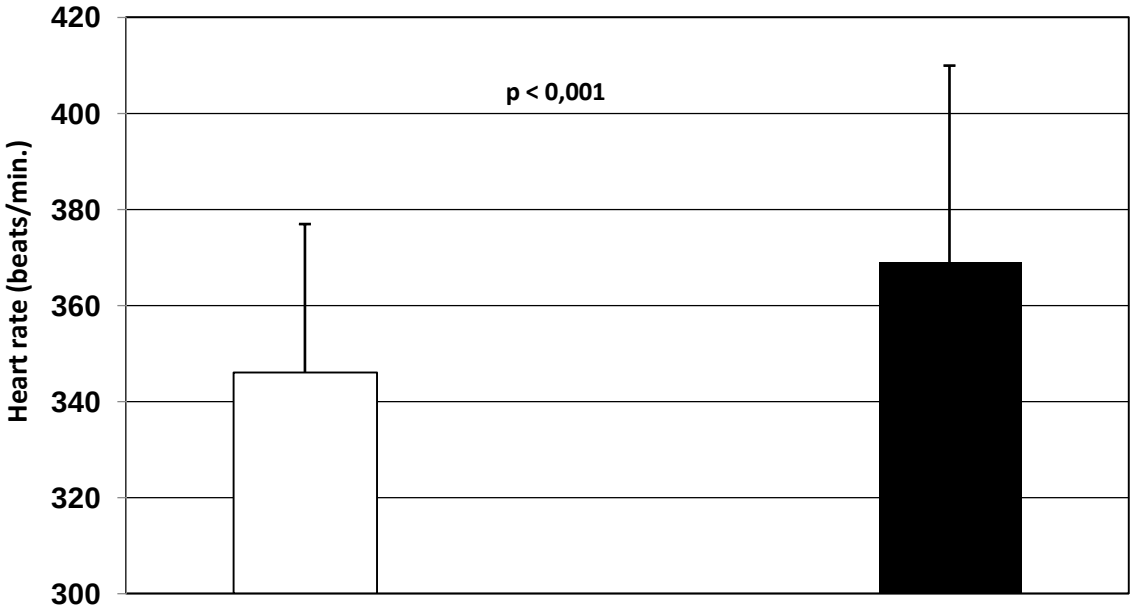

Figure 2 : Heart rate after adminsitration of pentobarbital anaesthesia during the light (open bar) and dark (black bar) parts of the rat regimen day. Data presented as mean ± SD. p<0,01 was considered to be a statistically significant difference

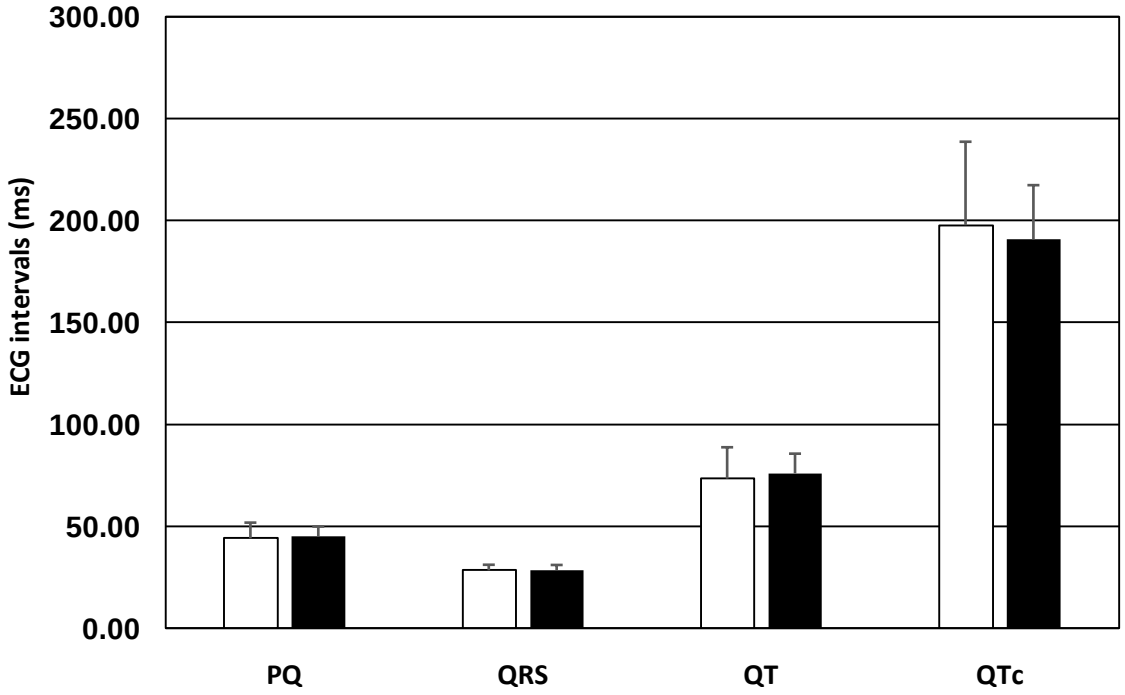

Figure 3 : Values of selected electrocardiographic (ECG) intervals during the light (open bars) and dark (black bars) periods of the regimen day in pentobarbital-anaesthetized rats. Data presented as mean ± SD

using a computer system (ECG Practic Veterinary, Prague, Czech Republic).

Acid-base balance and ion concentrations

Acid-base balance and ion concentrations were analyzed from blood samples obtained from the femoral artery.

Statistical analysis

Data are presented as mean ± SD. A non-paired *t* test was used for statistical evaluation; p<0,05 was considered to be statistically significant. The experiments were performed over the course of one year and the results were averaged independent of seasons.

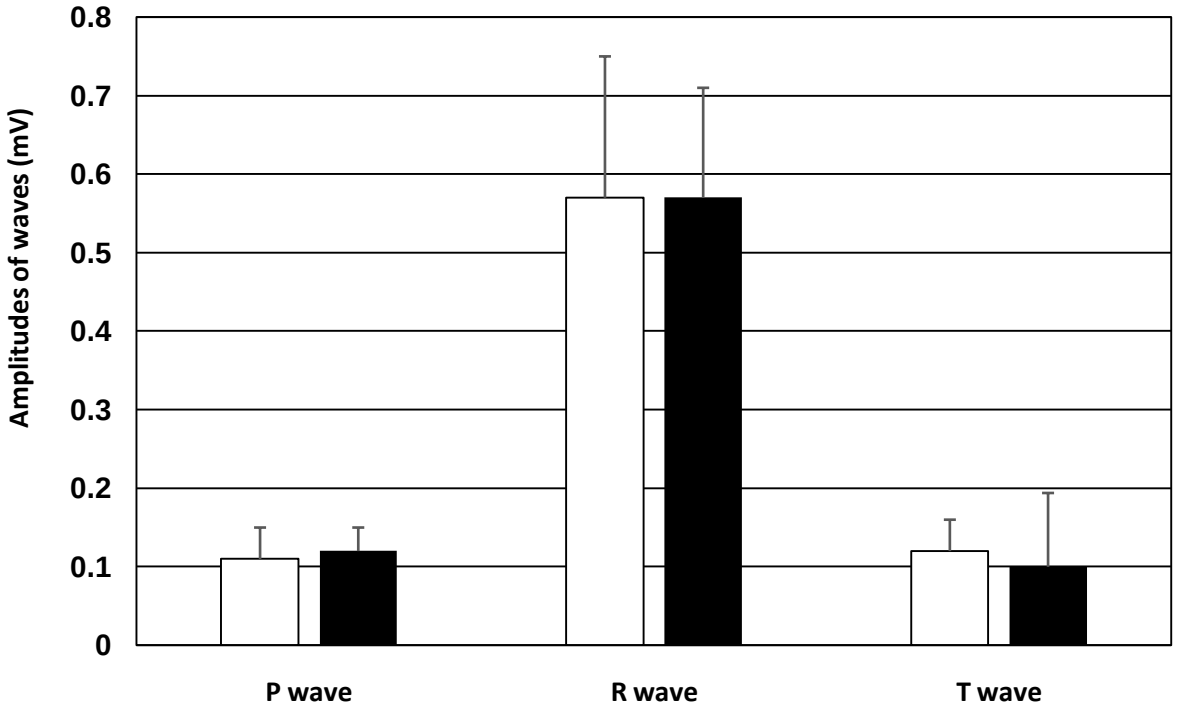

Figure 4 : Values of selected vawe amplitudes from electrocardiography during the light (open bars) and dark (black bars) parts of the regimen day in pentobarbital-anaesthetized rats. Data presented as mean ± SD

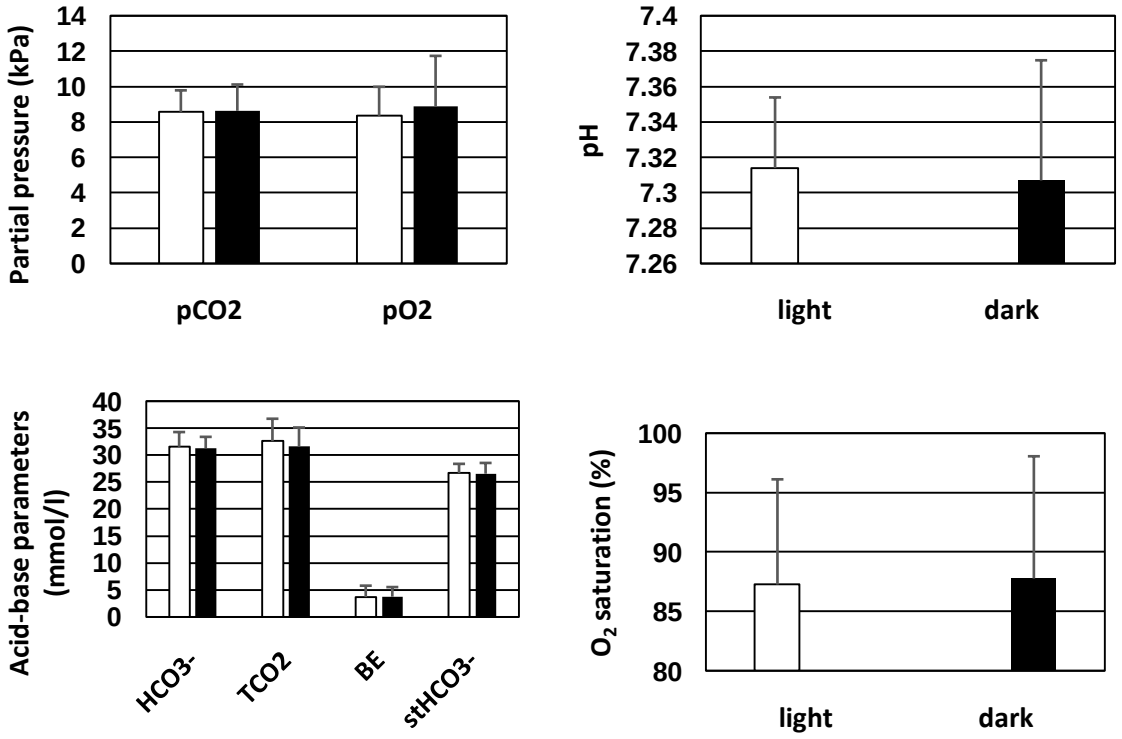

Figure 5 : Values of selected parameters of acid-base balance in arterial blood during the light and dark parts of the regimen day in pentobarbital-anaesthetized rats. Data presented as mean ± SD. BE Base excess; $stHCO_3^-$ Standard HCO_3^-; TCO_2 Total CO_2

RESULTS

Rectal temperature (light: RT1 36,693±0,487°C versus RT2 35,376±0,669°C; dark: RT1 36,995±0,621°C versus RT2 35,455±1,024°C) was significantly decreased under pentobarbital anaesthesia in light as well as in the dark period ($p<0,001$), with the preservation of significant LD differences only for RT1. The loss of LD differences was observed for RT2 (Figure 1).

In pentobarbital-anaesthetized animals, a significant LD difference ($p<0,001$) was found for heart rate (light group 346±31 beats/min versus dark group 369±41 beats/min) (Figure 2).

No significant LD differences were found for PQ, QRS complex, QT, QTc intervals (Figure 3) and for amplitudes of P, R and T waves (Figure 4).

LD differences were not found for any parameter of acid-base balance (Figure 5) or arterial plasma ion concentrations (Figure 6). LD differences with borderline statistical significance were found only for Na^+ ion concentration ($p < 0,05$), with higher levels during the light (ie, nonactive) part of the rat regimen day.

DISCUSSION

Despite of the limitations (absence of reference data from nonanaesthetized rats for comparison and relatively large dispersion of measured values, what is a problem typically encountered in *in vivo* studies), we found that rectal temperature showed significant LD differences before pentobarbital administration; however, these differences were eliminated after 15 min. from anaesthetic agent administration. This disruptive effect can be put in acute hypoxia because spontaneously breathing rats were in a systemic acidosis, hypoxia and hypercapnia in the light as well as in the dark period of the regimen day (our results and 17,18,19,20,21). However, studies involving ketamine/xylazine or zoletil anaesthesia demonstrated that, although rats were in the acute hypoxic state after ketamine/xylazine or zoletil administration, LD difference in rectal temperature was preserved (22,23). We speculate that the loss of the LD difference can be the specific pentobarbital effect.

Pentobarbital does have not propably so significant cardiodepressive effect as it is described by more authors (5,3,4). In nonanaesthetized rats, the circadian rhythm in heart rate exhibited higher values during the dark (active, mean 355±8 beats/min) compared with light (nonactive, mean 315±6 beats/min) part of the regimen day, as reported by Molcan et al. (24,25). In the present study, heart rate was not depressed and significant LD differences were preserved.

LD differences in electrophysiological parameters of atrial and ventricular complexes were eliminated when the animals were under the influence of pentobarbital anaesthesia. This suggests that myocardial vulnerability to ventricular arrhythmias originating from disorders of production and conduction of impulses, and from disorders of refractory period dispersions, are likely independent of the LD cycle. Understanding the electrophysiological properties of the heart is vital to the recognition of causes of various types of ventricular arrhythmias. These properties also appear to be dependent on the time of day experiments are performed (26,27).

CONCLUSION

Pentobarbital anaesthesia (likely associated with hypoxia) disturbs or, at least, modifies the daily rhythmicity of electrophysiological ECG parameters. Following pentobarbital anaesthesia, the animals exhibited systemic acidosis, hypoxia and hypercapnia in the light as well as in the dark period of the regimen day; LD differences in acid-base balance and ion concentrations were not apparent. Therefore, pentobarbital is probably not an ideal anaesthetic agent for chronobiological studies investigating in vivo electrophysiological myocardial properties in rat models.

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