Chapter 7

Chronobiology of Acid-Base Balance under General Anesthesia in Rat Model

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Abstract

The design and development of experimental, in vivo, chronobiological animal models may help reveal some of the relationships between circadian rhythms and biological functions. In vivo experiments require the use of appropriate anesthesia, which should be selected according to their particular effect on the organism. The aim of study was to review the status of acid-base balance and ion concentration in arterial blood under common used general anesthesias in experiments in dependence on the light-dark (LD) cycle in spontaneously breathing rats. The experiments were performed using 3- to 4-month-old pentobarbital(P)-, ketamine/xylazine(K/X)-, and zoletil(Z)-anesthetized female Wistar rats after a 4-week adaptation to an LD cycle (12 h light and 12 h dark). We concluded that P anesthesia disturbs LD dependence of acid-base balance compared to K/X and Z anesthesia, but LD differences in plasma ion concentrations are disturbed under all type of general anesthesia. P anesthesia is not the most appropriate type of anesthesia in rat chronobiological experiments. It eliminated LD differences and also produces a more acidic environment, more pronounced hypercapnia and hypoxia than K/X and Z anesthesias. This should be taken into account because the altered internal environment may affect the activity of systems whose functions are primarily dependent on acid-base balance.

Keywords: Chronobiology, electrophysiology of the heart, general anesthesia, internal environment, rat

1. Introduction

At the end of the eighteenth and early nineteenth century, the white rat became the most commonly used experimental animal in biomedical research because it was recognized as the preeminent model of the mammalian system. Currently, rat models are widely used not

only because of their low costs but also for their ability to mimic several human pathologies. These models are used to analyze basic physiological mechanisms, for preclinical and toxicological studies and/or the evaluation of therapeutic approaches [1, 2]. Rats are also useful model animals for studying acid-base balance, especially in relation to the cardiovascular and respiratory systems [1].

The design and development of experimental, in vivo, chronobiological animal models may help reveal some of the relationships between circadian rhythms and biological function, which is sometimes exceedingly difficult to study in humus. Popilskis et al. [3] referred to the fact that "nonhuman primates are important models for a wide variety of biomedical and behavioral research because of their close phylogenetic relationship to humans and they are useful models for experimental surgical studies." However, in the design and development of such chronobiological in vivo rat models, several problems may be encountered. First is the fact that homeostatic regulatory mechanisms are not eliminated; therefore, the responses of the animal as a whole are only a reflection of these mechanisms at a particular time of day. Second is that the circadian rhythms of the observed function itself are not accounted for. Finally, the initial state of the internal environment and the parameters of the function being observed—after the induction of general anesthesia—are often not considered.

In vivo experiments require the use of appropriate anesthesia, which should be selected according to their particular effect on the organism. Moreover, an increasing number of rat and mice studies have acknowledged that the toxicity and efficacy of some anesthetic agents fluctuate in circadian dependence. For example, the toxicity of barbiturates is higher in the early morning [4], and mortality after halothane anesthesia moves from 5% during the day to 76% at night [5]. The toxicity of althesin is highest around 10:00 h [6], and the effective time of althesin anesthesia is 20% longer at 12:00 h than at 06:00 h [5]. Nevertheless, anesthesia has played an important role in ensuring humane surgical/interventions in experimental animals, particularly in long-term in vivo protocols requiring animal survival. Presently, anesthetic practice is primarily based on physiology. The importance of the application of physiological principles in anesthesia has been reaffirmed and emphasizes the need for progress in systemic physiology [7].

2. Acid-base balance, anesthesia, and circadian rhythms

To survive, all living organisms need to maintain acid-base balance and oxygenation. The key role of homeostatic maintenance in all living organisms is not at odds with the observation that various biological parameters are dynamic. Rhythmic changes observed in humans that occur regularly play an important role in adaptation to dynamic environments. Chronobiology affects the activities and functions of the organs and tissues and is also a driver of anatomical, physiological, and molecular changes [8]. Control of acid-base balance depends on the concentration of H^+ and HCO_3^- ions in bodily fluids. In healthy wakeful mammals, including humans, compensatory mechanisms exist for the maintenance of the acid-base balance necessary for normal enzymatic activity, electrolyte diffusion, hemoglobin saturation, and heart contraction, all of which leads to normal functioning of vital organs [9].

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The problem of acid-base balance in anesthesia was addressed by several authors in the early decades of the twentieth century. It was then pointed out that patients under general anesthesia experienced metabolic acidosis due to the ineffective metabolism of carbohydrates in states of unconsciousness [10]. However, later works began to report that this acidosis has a respiratory origin due to disordered respiration [11, 12]. In 1955, Lucas and Milne [13] highlighted the respiratory origin of acidosis in 166 patients who underwent surgery. Respiratory acidosis has been shown to be detrimental during surgery, because it predisposes to shock and the occurrence of problem reflexes. It has been shown that in deep general anesthesia with spontaneous breathing, respiratory acidosis invariably occurs regardless of the anesthetic used. If controlled breathing is used, significant respiratory alkalosis is common with a normal arterial CO_2 pressure of approximately 20 mmHg. For anesthesiologists, metabolic acidosis associated with hypothermia and circulatory arrest is particularly important in cardiac and peripheral vascular surgery [14]. Monitoring of acid-base balance is recommended, especially for prolonged surgical procedures. There are studies indicating that patients undergoing inhaled anesthesia are affected by metabolic acidosis, which depends not only on the duration of the operation but also on the duration of anesthesia. As the duration of general anesthesia is prolonged, pH decreases significantly [15, 16]. This most likely also applies to animal models involving general anesthesia. Therefore, the choice of anesthetic and its effect on the respiratory and cardiovascular system is critical [17, 18].

Changes in the functional efficiency of these systems lead to changes in acid-base balance, and vice versa, changes in acid-base parameters affect the functional state of these systems. Similarly, changes in acid-base balance also reflect 24 h fluctuations in respiratory and cardiovascular functions. Therefore, reference values for acid-base balance can cause problems because the parameters of acid-base balance and ion concentration reflect the current state of the organism at a given time. Results are often compared with average reference values and often regardless of their dependence on the circadian rhythm.

However, rats are typical night animals, which adapt to a natural or controlled artificial light-dark (LD) cycles, which are the strongest synchronizers of endogenous rhythms. This means that their physiological functions exhibit circadian rhythmicity (i.e., fluctuate over a 24 h period).

If we focus on the respiratory system, data confirm that ventilation and metabolism in rats exhibit circadian rhythms and rebut the hypothesis that breathing is affected only by the current state of wakefulness or sleeping. The effects of circadian rhythms on breathing in sleep and wakefulness, as well as the rate of metabolism, are additive in the rat [19]. Some measures that reflect the mechanical properties of the lungs, such as functional residual capacity, forced expiratory volume, and respiratory airways resistance, vary periodically with the time of day. Additionally, resting pulmonary ventilation, tidal volume, and respiratory rate are governed by circadian patterns. Circadian oscillations of the respiratory pattern occur independently of the daily rhythms of other activities or states of wakefulness or sleep. Recent measurements of breath patterns over an extended time period in intact animals have shown that circadian changes occur in a close time phase with changes in oxygen consumption, carbon dioxide production, and body temperature. However, none of these variables can fully explain the circadian pattern of breathing, the origin of which remains unclear [20]. Selected parameters

of the cardiovascular system (e.g., heart rate, blood pressure) in rats also demonstrate circadian rhythmicity [21, 22], which are regulated by various mechanisms, including those part of the autonomic nervous system [23, 24]. Vulnerability of the rat myocardium to ventricular arrhythmias during normal pulmonary ventilation demonstrates a defined 24-h course, with higher vulnerability during the light period of the day. The acrophase, calculated using the population cosinor test, was 22:53, with a confidence interval from 19:20 to 00:28 [25].

The problem of circadian variation of acid-base balance parameters, therefore, remains. Circadian rhythms of acid-base balance and blood gases have been studied in humans, and the following acrophases were found: pH at 16:05; stHCO_3^- at 18:45 h; HCO_3^- at 22:55 h; buffer bases (BB) at 19:03; pCO_2 at 2:47 pm; pO_2 at 04:39 h; HbO_2 08: 07 h; and Hb at 2:16 pm [26]. In rats placed in constant darkness, diurnal rhythms were found in glycemia, pH, and pCO_2. Light pulses of 30 min duration increased blood glucose levels but did not affect plasma pH and pCO_2. These circadian rhythms are most likely under the control of the suprachiasmatic nuclei in the hypothalamus, while the hyperglycemic reaction to light is not controlled by circadian clocks and, thus, may involve retinal inputs to areas of the suprachiasmatic nuclei that are not sensitive to visual inputs [27].

3. Ion concentrations, anesthesia, and circadian rhythms

Ion concentrations neither can be neglected nor is there question whether they are affected by anesthesia or whether their circadian rhythm is maintained under anesthesia. These states can change significantly, for example, in myocardial excitability, which also changes over a 24 h period and is dependent on ion distribution. Based on ion status in the body and their particular role, especially in electrophysiological processes occurring in vital tissues, determination is essential. Potassium, for example, is an essential mineral micronutrient and is the primary intracellular ion for all types of cells, providing vital maintenance of fluid and electrolyte balance in humans and animals [28, 29].

There is clear evidence of the presence of circadian rhythm in potassium and sodium concentrations [30–35]. In all the examined species in which these rhythms occur, overlap of the peak excretion of potassium and sodium occurs essentially at the same time during a 24 h period. It is assumed that the peak of sodium excretion corresponds to reduced sodium reabsorption, and the peak in potassium concentration corresponds to an increase in potassium secretion. Studies involving squirrels, monkeys [36], and rats [37, 38] indicate that cyclic changes in potassium excretion are independent of changes in plasma potassium concentration. However, the correlation between plasma potassium and cyclic potassium excretion has been observed in humans [39]. Maintenance of stable plasma potassium ion (K^+) concentration is extremely important because K^+ controls muscle and nervous activity. In humans, urinary excretion of K^+ peaks in the early morning (05:30–07:30 h), with a minimum at night (21:00–05:30 h) [40]. Circadian rhythmicity has also been demonstrated in thoroughbred racehorses, in which plasma K^+ exhibited a significant rhythm, with acrophase during dark periods [41]. Similar results were found in plasma K^+ concentration in mice, in which based on measurement of urinary excretion, investigators found that peak excretion occurred in the resting

period [42]. Circadian variation of plasma sodium ion (Na^+) in the rat was also demonstrated in a study by Sotak et al. [43]. Electrogenic Na^+ transport in the rat colon was significantly higher during the subjective night than during the subjective day. Transporters and channels operating under the control of NaCl absorption exhibit diurnal regulation, and the role of the intestinal clock in coordinating intestinal NaCl absorption is presumed.

Because the above described events occur primarily in the kidneys, renal function is influenced by circadian clocks through two types of circadian inputs. The first is onset of renal rhythms through external circadian signals such as rhythms of hormones, food intake, activity, and body temperature. The second is the activity of the internal renal circadian clock. For example, Doi et al. [44] reported that the circadian time system controls the reabsorption of sodium in the distal nephron and in the collecting channel via the effect of aldosterone production in the adrenal glands. On the other hand, Rohman et al. [45] reported that internal renal clocks directly regulate Na^+/H^+ activity in the proximal tubule. Gumz et al. [46] reported that the circadian repressor period 1 is able to regulate expression of epithelial sodium channels in the cells of the collecting channel. A study by Roelfsema et al. [47] reported that the maximum excretion of potassium, phosphate, and magnesium is only slightly affected by the dietary regimen, indicating that it depends mainly on endogenous rhythm. In contrast, the minimum excretion of these ions is determined by food intake. Maximum calcium levels, as well as minimal excretion, correlate with dietary regimen. The sodium excretion pattern differs from the calcium, potassium, phosphate, and magnesium patterns, indicating that it is controlled by another mechanism. Unless this fact is taken into account, we can encounter distortions in which the final results are interpreted from a state that does not correspond with the physiological state before administration of the anesthetic.

Sodium ions are necessary for the generation of nerve impulses and for the maintenance of electrolyte and fluid balance. In animals, sodium ions are necessary for these functions and for heart activity and certain metabolic functions [28]. Symptoms of hyponatremia can vary from none to severe [48, 49]. Mild symptoms include a decreased ability to process information, headaches, nausea, and poor balance [50]. Severe symptoms include confusion, seizures, and coma [48, 49]. Hypernatremia can evoke a strong feeling of thirst, weakness, nausea, and loss of appetite [51]. Severe symptoms include confusion, muscle twitch, and bleeding in or around the brain [51, 52].

Calcium ions also play a vital role in the physiology and biochemistry of organisms and the cell. They play an important role in signal transduction pathways [53, 54], where they act as a second messenger in neurotransmitter release from neurons, in the contraction of all muscle cell types and in fertilization. Many enzymes require calcium ions as a cofactor, those of the blood clotting cascade being notable examples. Extracellular calcium is also important for maintaining the potential difference across excitable cell membranes, as well as proper bone formation. Symptoms of hypercalcemia may include abdominal pain, bone pain, confusion, depression, weakness, kidney stones, or abnormal heart rhythm and cardiac arrest [55]. Hypocalcemia can be associated with disorders of hemocoagulation, numbness, muscle spasms, seizures, confusion, or cardiac arrest [56]. Chloride is an essential electrolyte located in all bodily fluids and is responsible for maintaining acid-base balance, transmitting nerve impulses, and regulating fluid in and out of cells.

What is the effect of anesthetics on ongoing ion-dependent processes? Evidence from voltage-clamp studies of individual nerve fibers suggests that, for example, molecules of local anesthetic interact with sodium channels directly from the inside of the nerve membrane. Anesthetics bind to sodium channels, which open during membrane depolarization and prevent normal sodium flow. Anesthetic molecules can separate from open channels, but not from channels that remain closed when the nerve is kept in the resting state. The "gate" properties, which regulate the opening and closing of sodium channels, are reversibly adjusted during anesthesia [57]. Despite the significant advances in chronobiological studies, the mechanisms of circadian regulation of ion channels remain largely unknown. By exploring and understanding the circadian regulation of the ion channel in detail, progress in the development of therapeutic effective strategies for the treatment of sleep disorders, cardiovascular diseases, and other diseases associated with circadian desynchronization [58] will be developed.

4. Chronobiology of anesthesia

Anesthesia is often required in in vivo experiments to ensure comfort and to eliminate pain in animals. However, in small animals, the use of anesthesia can cause certain problems, and therefore, it is necessary to recognize the effect of anesthesia on the internal environment and to account for LD changes in the individual parameters of homeostasis. However, from experimental practice, we know that experiments are performed mostly during working hours (i.e., during light). Thus, if rats are synchronized to the light and dark modes corresponding to the annual season, experiments are performed in the light period of their regimen day (i.e., during their inactive period, when many physiological functions are inhibited). Experiments are, therefore, essentially performed on "sleeping" animals, and questions regarding function during the active part of their regimen day will remain. However, most methodologies do not specify the time of day at which the experiments are performed or the factors responsible for changes in the particular monitored parameters over time. Instead, they focus primarily on current mechanical and metabolic changes, often regardless of the functional status of the body systems over a 24 h period, which may be a problem from a chronobiological point of view [59, 60]. Animal adaptation should, therefore, be taken into account, particularly in in vivo experiments.

Normative data regarding arterial acid-base balance and plasma ion concentrations would help to identify healthy animals suitable for experiments [1], and there are studies that have examined the reliability of these data [61]. **Tables 1** and **2** summarize the ranges of some acid-base balance parameters and ion concentrations in arterial rat blood, which have been described in several published studies. However, the time at which the experiments were performed or the time of blood sampling for evaluation of blood gases, pH, bicarbonates, and some ions, or the synchronization of animals to the LD cycle, was not considered in the methodologies of these studies.

Although chronobiological studies investigating the interactions between general anesthesia and circadian rhythms are scarce, they all suggest that general anesthesia has a significant

Author(s) (year of publication)	pH	pCO_2 (kPa)	pO_2 (kPa)	HCO_3^- (mmol/l)
Lewis et al. [62]	7.43	5.47	12.13	
Pepelko and Dixon [63]	7.446–7.486	5.24–5.74	11.77–12.71	
Brun-Pascaud et al. [64]	7.45–7.49	4.2–4.99	11.26–12.72	24–27
Girard et al. [65]	7.46–7.47	4.57–4.71	12.72–13.02	25–25.8
Hess et al. [66]	7.43–7.51	3.33–4.67	12.2–15.4	
Dettmers et al. [67]	7.38–7.46	5.19–5.99	9.4–11	
Chi et al. [68]	7.27–7.37	4.78–5.77	13.8–17	
Ohoi and Takeo [69]	—	4.66–5.32	13.3–17.3	
Schultz et al. [70]	7.35–7.45	3.33–5.32	10.6–14.6	
Sun and Wainwright [71]	7.40–7.45	4.64–5.32	11.3	
Forkel et al. [72]		5.16–6.39	12.85–15.48	
Valenza et al. [73]	7.41–7.43	5.18–5.48	—	25.3–27.1
Subramanian et al. [1]	7.26–7.4	5.05–7.51	10.76–14.60	21.5–28.1
Peralta-Ramírez et al. [74]	7.2–7.46	5.62–6.20	—	23.2–25.8
Luo et al. [75]	—	5.58–6.08	10.37–12.19	
Range*	**7.369–7.452**	**4.75–5.298**	**10.75–14.184**	**23.8–26.78**

*Ranges were calculated as the mean value from the lower and upper limits of the ranges reported in these studies.

Table 1. Values of pH, blood gases, and bicarbonate in the arterial blood of rats published in previous studies.

Authors (year of publication)	Na^+ (mmol/l)	K^+ (mmol/l)	Ca^{2+} (mmol/l)	Cl^- (mmol/l)
Menegon et al. [76]	142.1–143.9	3.6–3.8		
Costa et al. [77]	138.9–141.1	4.74–4.86	6.72–7.38	
Valenza et al. [73]	132.4–140	4.1–4.42		102.9–107.7
Subramanian et al. [1]	140.7–145.6	3.08–4.02		
Peralta-Ramírez et al. [74]	134.6–137.3	3.93–4.25	1.23–1.29	104.4–108.1
Range*	**137.4–140.7**	**3.86–4.21**	**?**	**103.7–107.9**

*Ranges were calculated as the mean value from the lower and upper limits of the ranges reported in these studies.

Table 2. Arterial plasma ion concentrations in the arterial blood of rats according to previously published studies.

effect on biological functions [78]. Some have pointed to the temporal dependence of some anesthetic effects on the [78] circadian rhythm. For example, in locomotor activity, a phase shift of circadian rhythm occurred after administration of selected anesthetics, indicating its dependence on time. Pentobarbital injections induced both advanced and delayed phase shifts in the circadian rhythm of movement activity in SK mice; however, no phase shifts were observed in any circadian time with pentobarbital injections in C57BL mice. This suggests

that differences in phase shifts after the use of pentobarbital are not quantitative but qualitative [79], and that pentobarbital-induced phase shifts are not the result of increasing levels of activity [80].

In a study by Pang et al. [81], pentobarbital had no apparent effect on melatonin release and did not affect plasma levels of cerebral natriuretic peptide in rats, in which both hormones are at a relatively low level at 02:30 h [82]. Naguib et al. [83] described the effects of anesthesia on melatonin production. Anesthesia disrupts the circadian rhythm of melatonin, the major humoral transmitter of suprachiasmatic nuclei activities in the hypotalamus [84–86]. It appears that intravenous anesthetics with different behavioral profiles act on different and specific ligand-bound ion channels to create specific anesthetic behavior. Whether the anesthetic effect of melatonin is due to a direct effect on melatonin receptors remains largely unknown. Melatonin receptors, as such, are not commonly considered to be molecular targets for general anesthetic effects. However, there is evidence to suggest that the central effects of melatonin include at least partial facilitation of GABAergic transmission by modulation of GABA receptors [87–89]. In a study by Mihara et al. [90], pentobarbital demonstrated no effect on melatonin secretion or on movement activity, regardless of the time of dosing. On the other hand, in rats under general propofol anesthesia, the plasma concentration of melatonin decreased over the first 4 h after anesthesia induction and increased after 20 h. Thus, general propofol anesthesia abolishes the circadian rhythm of melatonin in rats adapted to an LD cycle [91].

Results of a study by Kana et al. [92], involving the inhalation anesthetic sevoflurane, reported that sevoflurane had the greatest efficacy in suppressing mPer2 expression (mPER2 acts as a positive rhythm transcription regulator in hypothalamic suprachiasmatic nuclei) in the morning. The investigators proposed that, in the morning, this biochemical reaction is inhibited by anesthesia, which can lead to suppression of mPer2 expression and effectively reflect circadian clocks. However, at the phase delay of movement cycle activation, sevoflurane acted independently of time.

Prudian et al. [93] and Pelissier et al. [94] reported a disrupting effect of ketamine on circadian rhythms; however, this effect was associated only with a modification of acrophase, amplitude or mesor, without loss of daily rhythmicity. To date, however, there is no literature evidence supporting the effect of general anesthesia on acid-base balance and ion concentration in arterial blood, depending on circadian rhythmicity or LD cycles. This highlights the fact that different anesthetics may have different effects on the circadian rhythms of many parameters.

5. Aims

The specific objective of the present in vivo study is to investigate chronobiological aspects of the status of acid-base balance and plasma ion concentrations in arterial blood (i.e., existence

of possible circadian variations) and to determine whether there are differences between types of anesthesia after the immediate application of the most common anesthetics in in vivo rat experiments, pentobarbital (P), ketamine/xylazine (K/X), and zoletil (Z) in spontaneously breathing rats.

6. Materials and methods

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 approved by the Ethics Committee of the Medical Faculty of Safarik University (Kosice, Slovak Republic) (permission numbers 2/05 and ŠVPS SR: Ro-4234/15–221).

The present study was performed using female Wistar rats (mean [±SD] weight 310 ± 20 g), 3–4 months of age after a 4-week adaptation to an LD cycle (12 h light:12 h dark [intensity of artificial illumination 80 Lux]; 40–60% humidity; cage temperature 24°C; two animals/cage; *ad libitum* access to food and water). 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 to 18:00 h. The effect of the dark period was monitored after adaptation to the inverse setting of the LD cycle (i.e., with the light period from 18:00 to 06:00 h) (**Figure 1**).

The animals were divided into one of three experimental groups according to anesthetic agent used (**Table 3**). Approximately 20 min after administration of anesthetic agent, the spontaneously breathing animals were fixed supine to an experimental table. pH and blood gases from blood samples obtained from the femoral artery were examined using a blood-gas analyzer

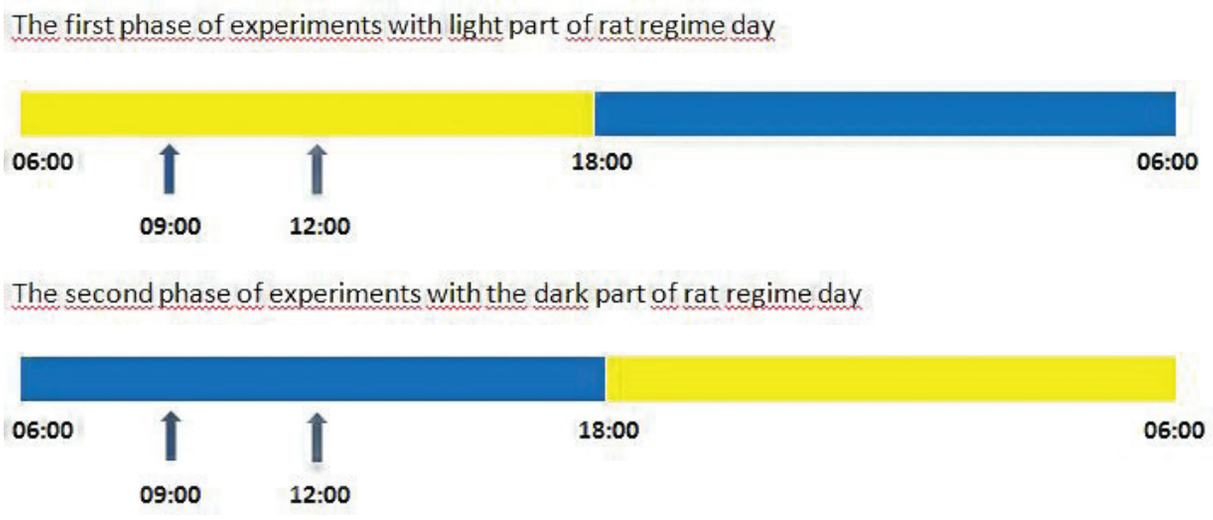

Figure 1. Scheme of adaptation to the light-dark (LD) cycle. Arrows indicate the time of the experiment. The experiments were performed once in each animal in the course of a single LD period (the first animal at 09:00 h and the second at 12:00 h).

	Experimental period	Number of animals	Anesthesia	Route of administration
Group 1	Light	16	*Pentobarbital* (40 mg/kg, SPOFA, Prague, Czech Republic)	Intraperitoneal
	Dark	27		
Group 2	Light	11	*Ketamine* (100 mg/kg, Narkamon) + *xylazine* (15 mg/kg, Rometar, SPOFA, Prague, Czech Republic)	Intramuscular
	Dark	13		
Group 3	Light	10	*Zoletil* (30 mg/kg, VIRBAC, France)	Intraperitoneal
	Dark	12		

Table 3. Experimental groups.

(ABL 800 Flex, Radiometer Medical, Copenhagen, Denmark) in the Department of Laboratory Medicine, Faculty Hospital Louis Pasteur in Kosice. The depth of anesthesia was estimated according to whether painful stimuli evoked noticeable motor or cardiovascular responses.

6.1. Statistical analysis

The data were analyzed using GraphPad InStat (GraphPad Software, USA) and presented as mean ± SD. ANOVA was used to detect significant differences within a single end point. The Tukey-Kramer test was used to identify significant differences between groups; $p < 0.05$ was considered to be statistically significant. The experiments were performed over the course of an entire year, and the results were averaged independent of season and estrous cycle.

7. Results

7.1. pH

Under P anesthesia, significant LD differences in arterial pH were not found, and values remained at the same levels. Under K/X ($p < 0.001$) and Z ($p < 0.001$) anesthesias, the pH was significantly higher in the dark (active) versus the light part of the rat regimen day (**Table 4**, **Figure 2**). In the light part of the day, the pH values reflect acidosis, compared with the range calculated from other authors (**Table 1**) in all types of anesthesia, and there was no significant difference between individual types of anesthesia. In the dark part of the day, mean pH values were significantly higher in K/X ($p < 0.05$) and Z ($p < 0.05$) anesthesias compared with P anesthesia. The pH was acidic under P anesthesia, from normal to alkaline under K/X anesthesia and from acidic to normal under Z anesthesia.

7.2. pCO_2

Significant LD differences in pCO_2 were found under K/X anesthesia but not under P and Z anesthesias (**Table 4**). In both light parts of the rat regimen day, significant hypercapnia

	Pentobarbital		Ketamine/xylazine		Zoletil	
	Light	**Dark**	**Light**	**Dark**	**Light**	**Dark**
Acid-base parameter						
pH	7.31 ± 0.04	7.31 ± 0.07	7.33 ± 0.04	7.43 ± 0.14**	7.32 ± 0.05	7.38 ± 0.06***
pCO_2	8.58 ± 1.22	8.64 ± 1.49	6.76 ± 1.84	2.88 ± 0.72**	6.75 ± 0.93	6.65 ± 1.11
pO_2	8.36 ± 1.64	8.89 ± 2.86	7.17 ± 0.37	10.75 ± 1.84***	10.06 ± 2.31	8.46 ± 2.08*
HCO_3^-	31.56 ± 2.73	31.31 ± 2.09	28.02 ± 4.57	15.55 ± 5.62***	25.28 ± 1.3	28.8 ± 2.11***
$stHCO_3^-$	26.67 ± 1.72	26.55 ± 2.01	24.16 ± 1.24	19.22 ± 5.14*	22.95 ± 1.37	27.0 ± 1.91***
$ctCO_2$	32.61 ± 4.15	31.66 ± 3.48	28.4 ± 2.69	15.91 ± 5.93***	22.58 ± 1.05	25.6 ± 2.23***
BE	3.66 ± 2.12	3.43 ± 2.38	0.06 ± 1.71	−5.23 ± 6.34*	−1.41 ± 1.58	2.21 ± 1.34***
BB	51.56 ± 2.23	51.13 ± 2.82	48.34 ± 1.71	42.44 ± 6.65*	46.39 ± 1.56	50.21 ± 1.34***
ctO_2	9.22 ± 2.29	10.28 ± 2.72	11.76 ± 5.11	20.05 ± 0.56*	18.58 ± 1.34	19.17 ± 1.05
$SatO_2$	87.25 ± 8.86	87.8 ± 10.34	84.66 ± 2.96	93.29 ± 7.42**	89.96 ± 5.46	89.25 ± 4.64

Data presented as mean ± SD.*$p < 0.05$;

**$p < 0.01$; and

***$p < 0.001$ statistically significant differences between the light and dark parts of the rat regimen day. pCO_2 (kPa) – partial pressure of carbon dioxide, pO_2 (kPa) – partial pressure of oxygen; HCO_3^- (mmol/l) – bicarbonate; $stHCO_3^-$ (mmol/l) – standard bicarbonate; $ctCO_2$ – the sum of carbon dioxide bound to hemoglobin and carbon dioxide dissolved in plasma; BE (mmol/l) – base excess; BB (mmol/l) – total buffer bases; ctO_2 – the sum of oxygen bound to hemoglobin and oxygen dissolved in plasma, $satO_2$ (%) – saturation of hemoglobin by oxygen.

Table 4. Values of acid-base balance parameters for selected type of anesthesia in the light and dark parts of the rat regimen day.

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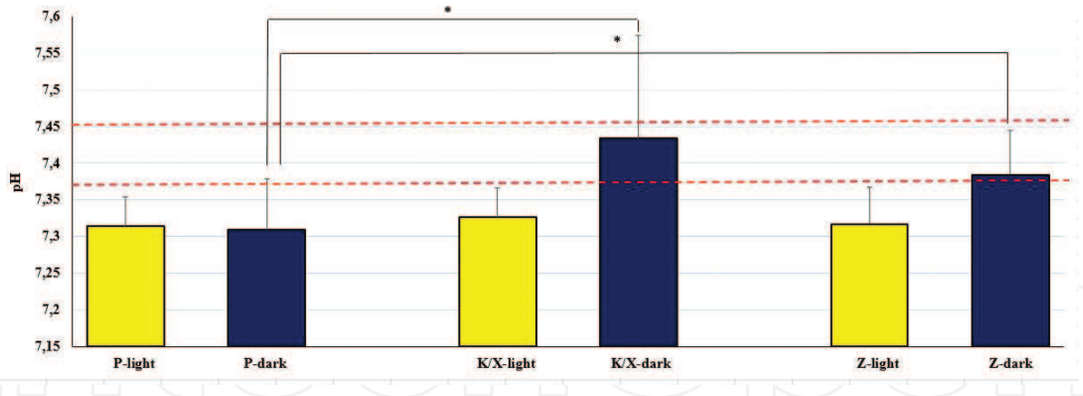

Figure 2. pH in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-, ketamine/xylazine (K/X)- and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. * $p < 0.05$ was considered to be a statistically significant difference between individual types of anesthesia. Red dotted lines represent the ranges reported in **Table 1**.

occurred under P and Z anesthesias. More pronounced hypokapnia was found under K/X anesthesia in the dark part. In the light part, there was a significant difference between P and Z anesthesia ($p < 0.001$), with higher values in P anesthesia. In the dark part of the rat regime day, significant differences between all selected types of anesthesia (P vs. K/X [$p < 0.001$]; P vs. Z [$p < 0.01$]; and K/X vs. Z [$p < 0.001$]) were observed (**Figure 3**). Because the pCO_2 ranges listed in **Table 1** are considered to be physiological compared with these ranges, the mean pCO_2 reported in this study is in the range of hypercapnia for each type of anesthesia in both light parts, except K/X anesthesia in the dark part of the rat day.

7.3. pO_2

Similar to pH, LD differences in pO_2 were only significant in K/X ($p < 0.001$) and Z ($p < 0.05$) anesthesias (**Table 4**). However, it is interesting to note that for all types of general anesthesia used in this study, hypoxia was detected in spontaneously breathing rats in both light parts of

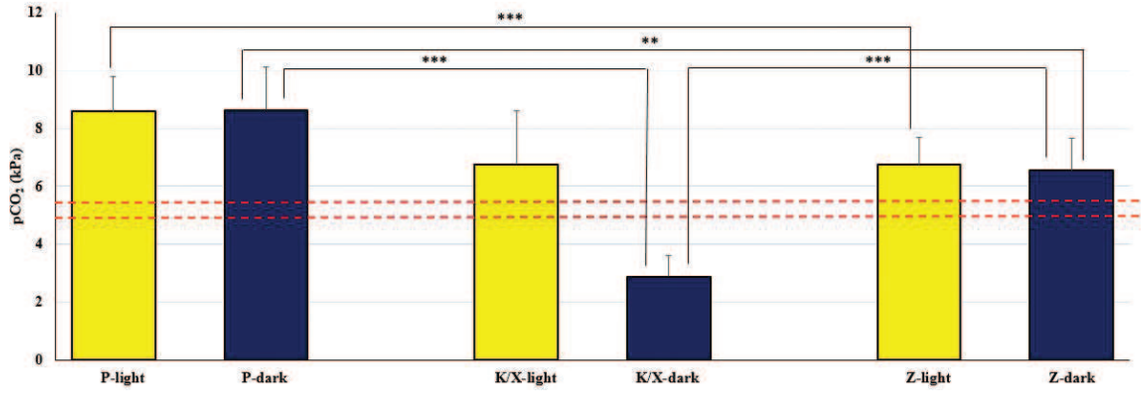

Figure 3. pCO_2 in the light (yellow columns) and dark (blue columns) parts of rat regimen day in pentobarbital (P)-, ketamine/xylazine (K/X)-, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. **$p < 0.01$, ***$p < 0.001$ were considered to be a statistically significant difference between individual types of anesthesia. Red dotted lines represent the ranges reported in **Table 1**.

the rat regimen day. Statistically significant differences were found in a light part between P and K/X ($p < 0.001$), P and Z ($p < 0.05$), and between K/X and Z anesthesia ($p < 0.001$), with the lowest values under K/X anesthesia. In the dark part, more pronounced hypoxia was under Z anesthesia ($p < 0.05$) compared with K/X anesthesia. Differences between P and Z anesthesias were not found (**Figure 4**).

7.4. HCO_3^-

Significant LD differences in HCO_3^- were detected under K/X and Z anesthesias (**Table 4**). Taking into account that the normal range of bicarbonate (from **Table 1**) is from 23.8 to 26.78 mmol/l, increased levels were measured in P anesthesia, which would correspond to metabolic alkalosis in both light parts of the regimen. Normal levels were detected in Z anesthesia in both light parts. In K/X anesthesia, the levels of HCO_3^- were dependent on the cycle of alternating light and darkness. Under this type of anesthesia, in the light part, values moved around the normal range; however, in the dark part of the day, levels were reduced to what corresponds to metabolic acidosis. Between individual anesthetics, significant differences were found, especially in the dark part of the rat regimen day (**Figure 5**).

7.5. BE, BB, and saturation of hemoglobin by O_2

Significant LD differences in total buffer bases (BB) and base excess (BE) were found in K/X and Z anesthesias (**Table 4**). BB moves from 40 to 60 mmol/l in all types of anesthesia and the BE from −8 to +12 mmol/l in both light parts of the rat regimen day under all types of anesthesia. Saturation of hemoglobin by oxygen was practically the same in all types of general anesthesia, and significant LD differences were not found except for K/X anesthesia, with higher saturation in the dark part of the rat regimen day. Significant differences of acid-base parameters between the single type of anesthesias are summarized in (**Table 5**).

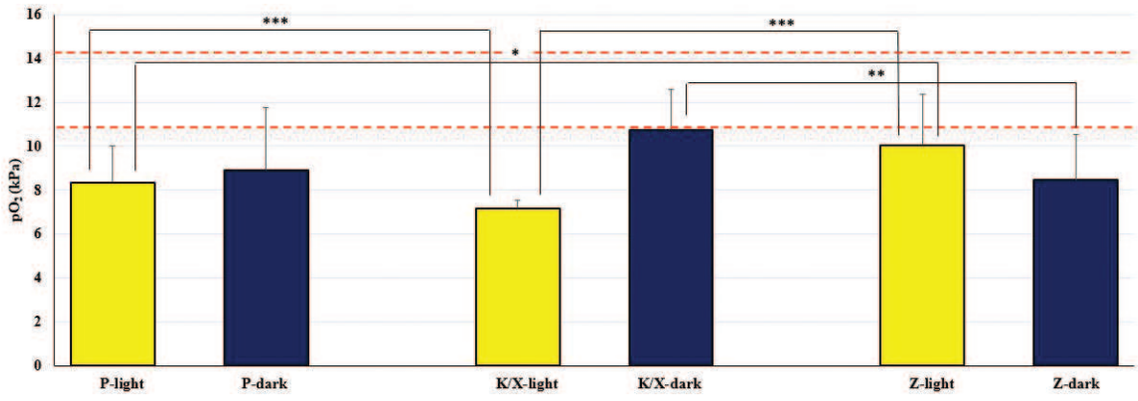

Figure 4. pO_2 in the light (yellow columns) and dark (blue columns) parts of rat regimen day in pentobarbital (P)-, ketamine/xylazine (K/X)- and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ were considered to be a statistically significant difference between individual types of anesthesia. Red dashed lines represent ranges reported in **Table 1**.

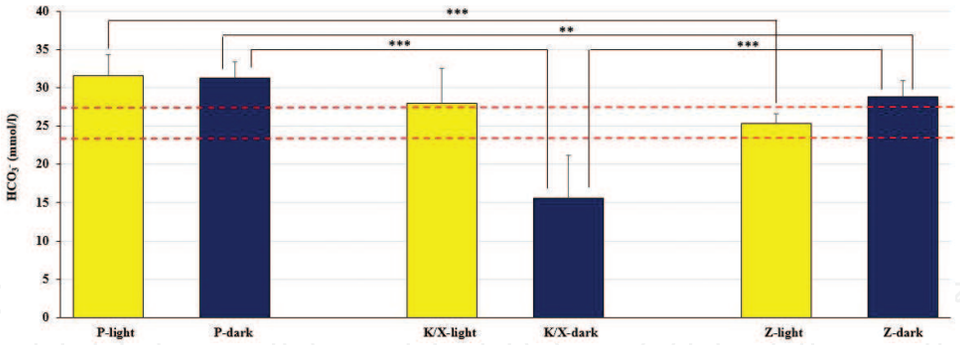

Figure 5. HCO_3^- in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-, ketamine/xylazine (K/X)- and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. **p < 0.01 and ***p < 0.001 were considered to be a statistically significant difference between individual types of anesthesia. Red dotted lines represent the ranges reported **Table 1**.

7.6. Ions

LD differences for plasma Na^+ concentration were not detected under any of the selected general anesthesias (**Table 6**). The highest Na^+ concentrations were under P anesthesia in the both light parts of the rat regimen day (light P vs. K/X, p < 0.01; P vs. Z, p < 0.01; dark P vs. K/X, p < 0.01; and nonsignificantly higher compared with Z anesthesia). In the light part of the day, the highest plasma concentration of Na^+ was recorded under P anesthesia and the lowest concentration in Z anesthesia but with increasing dispersion of values. Based on our findings, it appears probable that the distribution of Na^+ ions is significantly influenced by Z anesthesia (**Figure 6**). Under P anesthesia, regardless of the light or dark part of the day, hypernatremia was detected. In K/X and Z anesthesia, mean plasma Na^+ concentrations moved from hyponatremic to hypernatremic.

Significant (i.e., p < 0.01) LD differences in plasma K^+ concentration were found only under K/X anesthesia, with higher values during the dark part of the rat regimen day (**Table 6**).

	pH	pO_2	pCO_2	HCO_3^-	$stHCO_3^-$	BE	BB	$ctCO_2$	ctO_2	$satO_2$
Light										
P-K/X	0.617	**0.001**	0.094	0.166	**0.01**	**0.01**	**0.01**	**0.05**	0.339	0.419
P-Z	0.869	**0.05**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.401
K/X-Z	0.708	**0.001**	0.985	0.252	0.104	0.136	0.064	**0.01**	**0.05**	**0.01**
Dark										
P-K/X	**0.05**	0.137	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	**0.001**	0.251
P-Z	**0.05**	0.707	**0.01**	**0.01**	0.559	**0.001**	**0.001**	**0.001**	**0.001**	0.730
K/X-Z	0.268	**0.01**	**0.001**	**0.001**	**0.001**	**0.001**	**0.01**	**0.001**	**0.01**	0.119

Bold values indicate statistically significant differences. P – pentobarbital P; K/X – ketamine/xylazine Z – zoletil; pCO_2 (kPa) – partial pressure of carbon dioxide; pO2 (kPa) – partial pressure of oxygen; HCO_3-(mmol/l) – bicarbonate; $stHCO_3$ (mmol/l)-standard bicarbonate; $ctCO_2$ – the sum of carbon dioxide bound to hemoglobin and carbon dioxide dissolved in plasma; BE (mmol/l) – base excess; BB (mmol/l) – total buffer bases; ctO_2 – the sum of oxygen bound to hemoglobin and oxygen dissolved in plasma, $satO_2$ (%) – saturation of hemoglobin by oxygen.

Table 5. P values reflecting the statistical significance of differences in acid-base parameters among individual types of anesthesia in the light and dark parts of the rat regimen day.

Ion	P-light	P-dark	K/X-light	K/X-dark	Z-light	Z-dark
Na^+	145.08 ± 2.13	143.24 ± 1.7	140 ± 6.74	134.17 ± 5.56	133.97 ± 16.06	140.8 ± 7.67
K^+	4.69 ± 0.31	4.91 ± 0.30	6.81 ± 1.42	8.85 ± 1.31**	5.00 ± 0.71	4.68 ± 0.50
Ca^{2+}	1.31 ± 0.05	1.33 ± 0.05	2.14 ± 0.07	—	0.99 ± 0.44	1.00 ± 0.38
Cl^-	100.1 ± 1.21	100.51 ± 2.43	110.2 ± 2.39	—	104.8 ± 5.19	101.1 ± 5.1*

Data presented as mean ± SD.*$p < 0.05$,
**$p < 0.01$ statistically significant differences between the light and dark periods. P – pentobarbital P; K/X – ketamine/xylazine Z – zoletil; Na^+ – sodium, K^+ – potassium, Ca^{2+} – calcium and Cl^- chloride anions.

Table 6. Ion concentrations in arterial blood under individual types of anesthesia.

Under this type of anesthesia, the mean value was significantly higher ($p < 0.001$) compared with both P and Z anesthesias in both light parts of the day (**Figure 7**). Moderate hyperkalemia was detected under P and Z anesthesias in both light parts of rat regimen day.

Similar to Na^+, no significant LD differences in plasma Ca^{2+} concentrations were found (**Table 6**). Under P and Z anesthesias, plasma concentrations of Ca^{2+} were practically the same. In the light part of the day under K/X anesthesia, there was a significantly ($p < 0.001$) higher Ca^{2+} concentration versus P and Z anesthesias. In the dark part of the day under K/X anesthesia, the values were out of range of the ABL 800 Flex ion analyzer (**Figure 8**). Although significant differences were found between the different types of anesthesia in both light parts of the day, the animals were in relatively severe state of hypocalcemia, especially when under P and Z anesthesias.

A significant (i.e., $p < 0.05$) LD difference in plasma concentrations of Cl^- was found only under Z anesthesia (**Table 6**). Hypochloremia occurred under P anesthesia in both light parts of the rat regimen day. Normochloremia to hyperchloremia occurred under both K/X and Z anesthesias in both light parts of the rat regimen day (**Figure 9**). In the dark part of the day under K/X anesthesia, the values were out of the detection range of the ABL 800 Flex ion analyzer. Significant differences in ion concentrations between the single type of anesthesias are summarized in (**Table 7**).

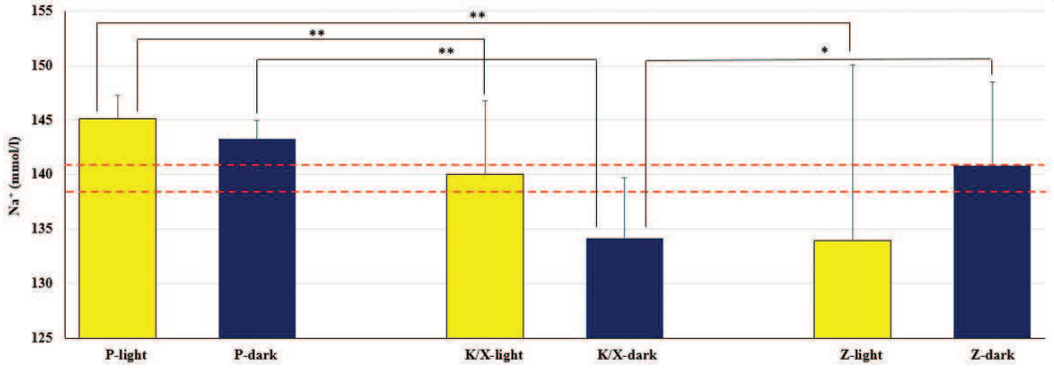

Figure 6. Plasma concentration of Na^+ in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-anesthetized, ketamine/xylazine (K/X)-anesthetized, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. *$p < 0.05$ and **$p < 0.01$ were considered to be a statistically significant difference between individual types of anesthesia. Red dashed lines represent ranges reported in **Table 2**.

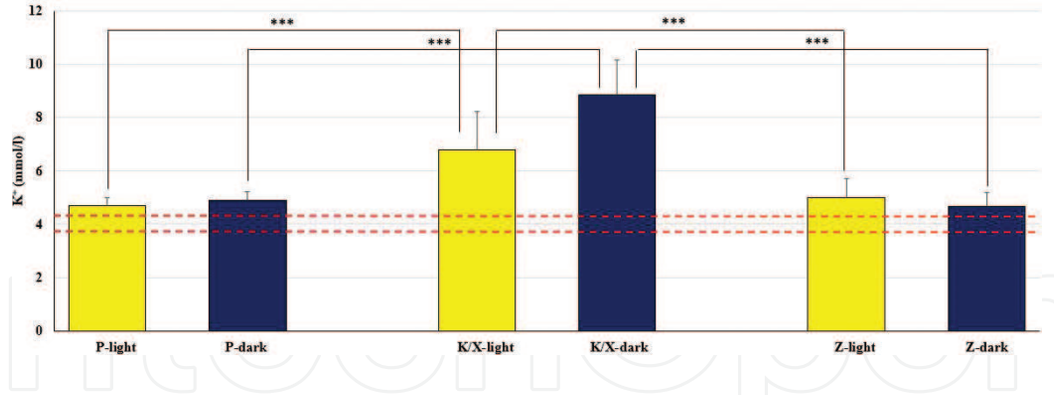

Figure 7. Plasma concentration of K^+ ions in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-anesthetized, ketamine/xylazine (K/X)-anesthetized, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. ***$p < 0.001$ was considered to be a statistically significant difference between single types of anesthesia. Red dotted lines represent the ranges reported in **Table 2**.

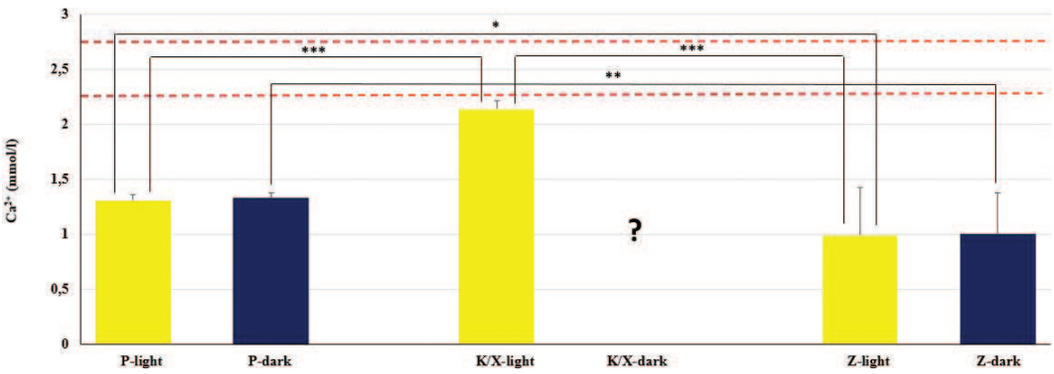

Figure 8. Plasma concentration of Ca^{2+} ions in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-anesthetized, ketamine/xylazine (K/X)-anesthetized, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ were considered to be a statistically significant difference between individual types of anesthesia. Red dashed lines represent ranges reported in **Table 2**.

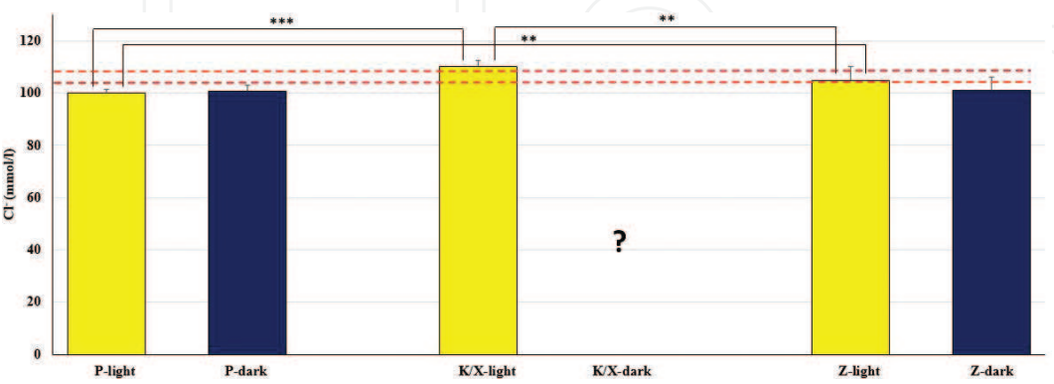

Figure 9. Plasma concentration of cl^- ions in the light (yellow columns) and dark (blue columns) periods in pentobarbital (P)-anesthetized, ketamine/xylazine (K/X)-anesthetized, and zoletil (Z)-anesthetized rats. Data presented as mean ± SD. ***$p < 0.001$, **$p < 0.01$ were considered to be a statistically significant difference between individual types of anesthesia. Red dashed lines represent the ranges reported in **Table 2**.

	Na^+	K^+	Ca^{2+}	Cl^-
Light				
P-K/X	**0.01**	**0.001**	**0.001**	**0.001**
P-Z	**0.01**	0.203	**0.05**	**0.01**
K/X-Z	0.202	**0.001**	**0.001**	**0.01**
Dark				
P-K/X	**0.01**	**0.001**	—	—
P-Z	0.246	0.770	**0.01**	0.687
K/X-Z	**0.05**	**0.001**	—	—

Bolded values indicate statistically significant differences.

Table 7. Differences in plasma ion concentrations of individual types of anesthesia in the light and dark parts of the rat regimen day.

8. Discussion

The methodological character of this study was based on the chronobiological perspective of the initial state in acid-base balance and plasma ion concentration in arterial blood after application of commonly used anesthetics in experiments, as well as to differences in parameters of the internal environment between used the selected types of general anesthesia. The methodical characteristics of this study highlight the potential risks of experimental design. Each of the acid-base balance parameters reflects the current state of the internal environment, which can significantly affect the functionality of the monitored system.

If we only hypothetically assume that experiments are performed during working hours (i.e., in the light [inactive], part of the rat regimen day), the values presented in **Tables 1** and **2** are comparable with our results only from the light (inactive) part of the day. In the dark (i.e., active) part of the rat regimen day, the values—although significantly different among the individual types of general anesthesia—may be within the normal range but can also move out of range; this also applies to ion concentrations. In this case, therefore, comparisons are irrelevant.

8.1. pH and blood gases

The cardiovascular system is particularly sensitive to changes in the internal environment. For example, earlier work by Gerst et al. [95] did not detect an impact of respiratory acidosis and alkalosis on the threshold of heart vulnerability to ventricular fibrillation in dogs; however, together with hypoxia, they increased its threshold [96]. Conversely, metabolic acidosis reduces the ventricular fibrillation threshold, reduces the maximum diastolic potential, shortens the duration of action potentials, inhibits excitability, stimulates impulse conduction between Purkinje fibers and muscle tissue [97], worsens atrioventricular (AV) conduction, and inhibits AV node automation [98]. Acidosis affects the mechanical and electrical activity of the

mammalian heart. In this way, acidosis can dramatically prolong the delay of AV conduction. In combination with short cycle times, this may cause partial or complete AV block of conduction and, consequently, contribute to the development of bradyarrhythmias under conditions of local or systemic acidosis [99]. Hypoventilation in rats is associated with systemic acidosis, hypoxia and hypercapnia, decreased mesor, amplitude, as well as altered circadian rhythm of ventricular arrhythmia threshold from one peak to two peaks, with a smaller peak between 15:00 and 18:00 h and higher between 24:00 and 03:00 h [25].

Our results indicate that P, K/X, and Z anesthesias cause acidosis, hypoxia, and hypercapnia, especially in the light period of the rat regimen day. In the dark part of the day, values are closer to physiological ranges, except for P anesthesia [100]. It also appears that differences in pH, pO_2, and pCO_2 differ among each type of general anesthesia, depending on the light period. The decrease in pH, observed in all types of anesthesia, is probably the result of a contemporaneous depression of pulmonary ventilation and decrease in body temperature in the light as well as in the dark part of the rat regime day.

We have confirmed the conclusions of other work investigating the effects of anesthesia on pulmonary ventilation. Induction of anesthesia in rats using P significantly increases pCO_2 and TCO_2, while pH is decreased [64, 101, 102]. P-induced anesthesia caused mild respiratory acidosis accompanied by an increase in arterial lactate levels. Urethane anesthesia leads to partially compensated metabolic acidosis. Hypothermia reduces metabolic acidosis and hypercapnia induced by P anesthesia. In urethane anesthesia, no difference was observed between hypothermic and normal values [103]. Alfaro and Palacios [104] compared acid-base balance in mildly hypothermic (30°C) and seriously hypothermic rats (20°C). The authors found that in the first group of hypothermic animals, respiratory alkalosis occurred with an increase in pH from 7.476 to 7.546 and a decrease in arterial bicarbonate from 22.9 to 16.8 mmol/L; in the second group, from 7.484 to 7.563 with a bicarbonate drop from 20.7 to 14.6 mmol/l. This pattern was clearly different in rats under P anesthesia (mild respiratory acidosis) and under urethane anesthesia (metabolic acidosis). Similar results were reported by Gaudy et al. [105]. Anesthesia may interfere with the development of processes that lead to the acid-base balance pattern observed in conscious animals. In 1997, Alfaro and Palacios [106] supplemented that their observations regarding the blood pH of normothermic anesthetized rats (body temperature Tb = 37°C) was also associated with an increase in plasma anions (lactate and Cl^-). More severe metabolic acidosis in rat blood were detected in urethane-induced hypothermia (Tb = 32°C).

Changes observed in rats anesthetized with the thiobarbiturate inactin were similar to urethane anesthesia, although they were generally less severe. Most subjects treated with barbiturates were significantly hypercapnic. Urethane anesthesia was characterized by a higher and more stable heart rate and greater pulse pressure. Arterial carbon dioxide and bicarbonate values in the urethane group were significantly lower at all sampling times than those obtained in the barbiturate groups [107]. In connection with hypercapnia, it is also interesting to note that mild hypercapnia increases peripheral tissue oxygenation in healthy individuals, which can improve resistance to infections after surgical intervention. Partial pressure of tissue oxygen, blood flow rate through the skin, cardiac index, and saturation of muscle oxygen increases linearly with partial CO_2 pressure. The observed difference in peripheral oxygenation is clinically important because previous work has suggested that a comparable increase in tissue oxygenation reduces the risk of infection from 7–8%, to 2–3% [108].

Considering changes in blood gases from a chronobiological perspective, Ohshima et al. [109] and Iwase et al. [110] reported interesting results regarding the effects of histamine on ventilation and the balance of energy metabolism via H1 receptors in the brain. The hypothesis was tested on mice as to whether the ventilatory response to hypoxia fluctuated between the light and the dark period and whether histamine H1 receptors are necessary for circadian variation. The results demonstrated that during hypoxic conditions, minute ventilation in wild type mice increased during the dark period. Hypoxia reduced metabolism, but O_2 consumption and CO_2 elimination were higher in the dark period. In H1 receptor knockout mice, changes in minute ventilation were minimal because minute ventilation was relatively increased with respect to O_2 consumption in the light period. In this group, HCO_3^- and BE were elevated in arterial blood, and serum levels of ketolate were increased, indicating metabolic acidosis. The results of that study assume that minute ventilation varies between the light and dark periods, and that H1 receptors play a role in the circadian variation of minute ventilation through acid-base balance control and metabolism in mice [109, 110].

Rectal temperature in rats measured before administration of anesthetic agent varies depending on the LD cycle, with significantly higher values in the dark (active) part of the day, indicating the preservation of the circadian rhythm of body temperature. After anesthetic administration, a significant drop in rectal temperature (rectal temperature before anesthetic administration versus rectal temperature 15 min after induction of anesthesia [$p < 0.001$]) has been observed under all types of anesthesia in both light parts of the rat regimen day [100]. Interestingly, LD differences in K/X and Z anesthesias were maintained, except for P anesthesia. These results confirm the well-known fact that thermoregulation is impaired under general anesthesia [111]. This basic process occurs when the body core temperature is redistributed to the surface of the skin by anesthetic-induced vasodilation and depression of hypothalamic thermoregulatory centers [112]. Thus, the loss of LD differences under P anesthesia confirms this fact, and that P likely also acts on the suprachiasmatic nuclei of the hypothalamus.

Sustained anesthesia and hypothermia may be required under certain conditions of critical care. Data suggest that mild hypothermia (35–33°C), in combination with sustained anesthesia, may reduce the need for high levels of breathing volume and respiratory rate without significant changes in arterial oxygenation and acid-base balance. The risk for barotrauma in ventilated rats exposed to conditions similar to critical care could, therefore, be reduced by using lower volume/pressure ventilation in the presence of mild hypothermia and P anesthesia [113]. Moderate hypothermia in rats induced by sustained P anesthesia reduces ventilation but without a change in arterial oxygenation or acid-base balance, measured at normal body temperature. In theory, observations in spontaneously breathing rats indicate that a combination of moderate hypothermia and anesthesia can be safely used to maintain adequate ventilation with relatively low ventilation. It is assumed that such a maneuver, when used during mechanical ventilation, can prevent secondary pulmonary damage by allowing a lower adjustment of the volume and pressure of the ventilator [114].

Metabolism and pulmonary ventilation change over a 24 h period and exhibit circadian fluctuations. Because their changes are always synchronic, blood gases can remain stable in a narrow range. Piccione et al. [115] monitored arterial blood gases, pH, body temperature and respiratory rate in 5 cows and detected a circadian rhythm only for pCO2. In cows, blood

gases remain highly stable for 24 h. Daily body temperature oscillations, respiratory rate, and probably many other factors affecting metabolism and pulmonary ventilation do not exclude excellent blood gas homeostasis.

If respiratory acidosis is induced after anesthesia, it is logical to adjust pulmonary ventilation so that the acid-base balance is adjusted to a physiological range. However, there is a problem with how to set up artificial ventilation to adjust acid-base balance parameters. The method of artificial ventilation for rats under general anesthesia has been in use since 1940 [116–119]. This can be a suitable procedure for creating experimental models observing the effect of pulmonary ventilation disorders on various functional systems. However, artificially controlled ventilation parameters using room air should be adequate to maintain acid-base balance. There are several types of normal artificial ventilation in rats that can be applied to maintain acid-base balance (**Table 8**).

The selection of anesthetic agent may be problematic with respect to the respiratory and cardiovascular systems [17, 18]. Changes in the functional performance of these systems lead to changes in acid-base balance. Conversely, changes in acid-base balance also reflect 24 h fluctuations in respiratory and cardiovascular function. Therefore, acid-base balance reference values may be problematic because acid-base balance only reflects the current state of the organism at a particular time of day. The results are then often compared with the average reference values, often regardless of dependence on the circadian rhythm of changes in acid-base balance. If both pH and partial pressures of the respiratory gases depend on respiratory and cardiovascular

Author (year)	Respiratory rate, breaths/min	Tidal volume, ml/100 g
Fagbeni et al. [120]	54	2
Richard et al. [121]	60	1
Guarini et al. [122]	55	2
Lott et al. [123]	70	1.5–2
Ohoi and Takeo [69]	40–60	1
Godin-Ribuot [124]	54	1.5
Oosting et al. [125]	60	3
Schultz et al. [70]	65–70	Not determined
Sun and Wainwright [71]	54	2
Häfner et al. [126]	30	Not determined
Tanno et al. [127]	44–55	1.5–2.5
Ravingerova et al. [128]	65–70	1.2
Wang et al. [89]	60–70	1.2
Neckař et al. [129]	65–70	1.2
Neckař et al. [130]	69	1.2

Table 8. Previously published artificial lung ventilation parameters to maintain normal acid-base balance ranges in vivo in rats.

http://dx.doi.org/10.5772/intechopen.75174

activities and demonstrate circadian rhythmicity in these systems, acid/base balance parameters will also exhibit a parallel circadian rhythmicity. The functional efficiency of the respiratory and cardiovascular systems is greater during periods of activity; therefore, pO_2 will also be higher at these times, and CO_2 output will be increased. pH depends on changes of pCO_2. The question, therefore, remains: to what extent are changes in acid-base balance parameters still acceptable in in vivo rat models? Additionally, to what extent should the dependence on circadian rhythms be accounted for in the design of in vivo experiments involving general anesthesia?

8.2. Acid-base balance and ion concentration

When considering parameters of acid-base balance, the most important is bicarbonate concentration. In general, given the impact of some processes on acid-base balance, it is advisable to especially consider changes in the concentrations of the major ions and their equilibrium to evaluate changes in the concentration of bicarbonate. The change in pH is secondary due to the change in the Henderson-Hasselbach equation. Eventual loss or addition of protons is immediately equalized by buffering mechanisms, and the capacity of which are significant with regard to regulating proton concentration.

Bicarbonate content in serum or plasma is a significant indicator of electrolyte dispersion and anion deficiency. Together with pH determination, bicarbonate measurements are used to diagnose and treat many potentially serious disorders associated with acid-base imbalance(s) in respiratory and metabolic systems. Concentration of bicarbonate reflects the acidity or alkalinity of the blood. In metabolic acidosis, the bicarbonate concentration is low, and in metabolic alkalosis, bicarbonate concentration is high. The actual concentration of bicarbonate reflects not only the metabolic component but also the respiratory component. For control of the respiratory component, standard bicarbonate is a better measure of the metabolic component than actual bicarbonate. Standard bicarbonate is inverse to the standard pH, which is pH under standard conditions (pCO_2 = 40 mmHg, temperature 37°C, and 100% oxygen saturation).

8.3. Bicarbonate and acid-base balance

The relationships between acid-base balance and ion management are closely connected. The main reason is that one part of the bicarbonate buffer has no charge (H_2CO_3 [i.e., CO_2]), while the second component is charged (HCO_3^-). Therefore, the bicarbonate anion must be in equilibrium with other ions to preserve electroneutrality in the internal environment. For partial pressure of CO_2, this does not apply, and therefore, its regulation can be largely independent. According to the Henderson-Hasselbach equation, the pH of the internal environment depends on the ratio of bicarbonate concentration to pCO_2. Regarding the regulation of most major ions (Na^+, K^+, Cl^-), these regulations are very sensitive but have only limited possibilities for rapid influence, resulting in serious functional consequences for the organism. In this case, if the concentration of a particular ion alters some pathological process, this change must be compensated by a change in the concentration of another ion to maintain electrical neutrality. Often, this compensation is afforded by changes in bicarbonate concentration. Biocarbonates, regardless of blood pH, alter the transcellular distribution of K^+, reflecting the utility of hydrogen carbonate therapy in hyperkalemia, even in conditions of compensated blood pH [131].

Our measurements indicated elevated levels of bicarbonate under P anesthesia, which, compared with the normal range (23.8–26.78 mmol/l in rats), would correspond to metabolic alkalosis, unless there were changes in other parameters of acid-base balance in both light parts of the day. However, under P anesthesia, we also found relatively severe acidosis, hypercapnia, hyperkalemia, and hypochloremia, which could signal the compensation of this state or the replacement of chlorides in the blood by bicarbonates. In this regard, P anesthesia induces more serious disruption of acid-base balance, independent of the cycle of alternating light and darkness. In K/X and Z anesthesias, these changes were more subtle, and when LD differences appear to be preserved, we assume that circadian rhythms are also preserved, and therefore, from a chronobiological point of view, these are appropriate types of general anesthesia.

8.4. BE and BB

BE relates to a true excess of base in the range (above or below) of the total BB. Normally, BB is 48–49 mmol/l. If BB is 40 mmol/l, it means that the buffer base was decreased by almost 8 mmol/l or BE is −8 mmol/l (also known as base deficiency). If BB is 60 mmol/l, it indicates that the base of the buffer is increased by approximately 12 mmol/l, or BE is +12 mmol/l. Fifty percent of BB is produced by bicarbonate and 25% by other buffers (proteins, phosphates, sulfates). In our experiments, BE and the total BB moved within the normal ranges, which would mean that buffering capacity was sufficient not only in the dark but also in the light period of the rat regimen day and under all types of anesthesia.

8.5. Ions

8.5.1. Potassium and acid-base balance

As early as the 1950s and 1960s, the relationship between extracellular potassium, bicarbonates, and blood pH was recognized. Relatively small changes in potassium concentration in the cell compartment can result in large changes in plasma potassium concentration. As a result, plasma potassium concentration may be reduced, normal, or elevated, despite normal stores of potassium in the body. The main regulator of transcellular potassium distribution is the pH of the extracellular fluid, which is reflected in blood pH. It was demonstrated that lowering the pH of blood increases serum potassium levels and vice versa [132–135]. It has recently been found that the concentration of extracellular bicarbonate—apart from its effect on extracellular pH—affects a wide range of metabolic reactions [136–139]. During this time, there was contradictory evidence that changes in blood hydrogen carbonate concentration in isohydric conditions alter plasma potassium concentration [140–143] in normokalemia, and no information regarding the role of bicarbonates in hypokalemia or hyperkalemia was available. At the increase of pH about 0.1, kalemia is increased about 0.5–0.6 mmol/l.

In acidemia, a number of "redundant" protons will enter the cells in which they will buffer. Consequently, a cation is transferred through the plasma membrane, which would in itself lead to a change in membrane potential. Instead of the proton, another cation is transferred from the intracellular to the extracellular space. Because the conductivity of the plasma membrane is highest for K^+ ions, primarily potassium ions will be transferred. Acidemia in this scenario leads to hyperkalemia. The total amount of potassium in the body does not increase,

and it only changes its distribution between compartments. From a whole-body perspective, potassium depletion will be a consequence of acidity, because its renal loss increases (so that heavier and longer-lasting acidosis will be accompanied by depletion of potassium at the current hyperkalemia). Similarly, alkalemia is accompanied by hypokalemia. However, the entire mechanism also works inversely: hyperkalemia causes acidosis and hypokalemia, on the other hand, leads to alkalosis. Simplified, we can imagine that potassium cations that move through the plasma membrane are exchanged for protons.

From the chronobiological point of view, however, this was not confirmed by our results. In each type of anesthesia, hyperkalemia was recorded, irrespective of whether the measurements were made in the light or dark part of the rat regimen day. Acidosis occurred only in the light part of the day under each type of anesthesia, while in the dark part of the day, the pH values also moved within normal ranges, but only under K/X and Z anesthesias These findings should, therefore, be taken into account to avoid application of particular anesthesias in the light part of the rat regimen day because positive correlations between pH and plasma K^+ concentration have been calculated for all types of anesthesia (P light r = 0.41, P dark = 0.16; K/X light r = 0.57, K/X dark r = 0.01, Z light r = 0.79, dark r = −0.22). What this means that the increase in plasma concentration K^+ shifts the pH to the alkalinity, respectively. Alkalosis increases K^+ leakage if the rat is in general anesthesia. In the dark part of the rat regimen day, no pH dependence on K^+ was found under all types of anesthesia.

If we generally consider the consequences of changes in plasma K^+ concentration affecting membrane processes, they touch primarily exciting tissues. In case of hyperkalemia, the concentration gradient decreases so that potassium escapes from the cell more slowly. However, the resting membrane potential becomes less negative and, therefore, in the initial phase of hyperkalemia, excitability increases (the resting potential is closer to the threshold). Increasing the potassium concentration in the extracellular environment by increasing the potential leads to blockage of voltage-gated Na^+ channels, and consequently, excitability decreases.

Considering the electrophysiology of the heart, hyperkalemia affects the production and conduction of impulses, which can lead to ventricular fibrillation through several mechanisms:

- the concentration gradient of K^+ in the direction from the intracellular into extracellular space is the key factor determining the value of the resting membrane potential. Increases in the extracellular K^+ concentration leads to a decrease in the gradient to a decreased outward K^+ current and thus to a decrease in the negativity of the membrane potential. In the myocardium, the resting membrane potential is reduced from −90 to −80 mV.

- at decreased negativity of the resting membrane potential, the difference between resting and threshold potential is lower and depolarization is more easily induced. If the negativity of the resting membrane potential continues to fall, the negativity of the threshold potential also begins to decrease.

- the value of the resting membrane potential also determines the number of sodium channels that open during depolarization to allow Na^+ input into the cell. The lower the negative resting membrane potential, the less the Na^+ channels are activated and depolarization occurs slower.

- repolarization is the result of opening K^+ channels and the subsequent outward K^+ current. For unclear reasons, the amount of K^+ from the cell paradoxically increases with increasing extracellular K^+ concentration. In hyperkalemia, therefore, acceleration of repolarization occurs.

Electrolyte abnormalities are becoming an increasingly important cause of arrhythmias. In humans monitored using electrocardiography, spiky and narrow T-waves (acceleration of repolarization) are the most common manifestations, QRS complex enlargement and prolongation of the PQ interval (slow depolarization). If hyperkalemia deepens, atrial activity may disappear, and ventricles are stimulated from AV node with resulting bradycardia. In severe hyperkalemia, the QRS complex expands, with consequent risk for ventricular fibrillation and cardiac arrest.

Although electrocardiographic (ECG) changes in hyperkalemic rats are poorly understood, it is clear that excess plasma potassium may also alter cardiac excitation. In addition, the effects of hyperkalemia on ECG in rats may differ from other species that do not have ST segments and longer QT intervals. At testing, the effects of two local anesthetics (bupivacaine and lidocaine) at normocalcemia and hyperkalemia were found that hyperkalemia with concentration 9.0 mmol/l had little effect on heart rate or AV conduction in the absence of bupivacaine or lidocaine. Nevertheless, the effect of local anesthetics on slowing the ventricular rate was significantly enhanced. For bupivacaine, ventricular deceleration to 50% vs. control, during hyperkalemia, was performed almost completely through inhibition of AV conduction whereas for lidocaine through not only inhibition of AV conduction but also atrial rate. Regardless of the mechanism, hyperkalemia of this grade increased the ventricular slowing effect of bupivacaine and lidocaine [144]. Kuwahara et al. [145] described changes in rat ECG in dependence on K+ levels. In moderate hyperkalemia, an increased amplitude of T wave occurred. The duration of the PR interval and the QRS complex was slightly reduced, and the P wave disappeared in most rats at potassium levels above 8.0 mmol/l. In advanced hyperkalemia (plasma potassium concentration higher than 7.5 mmol/l), conduction was suppressed in all parts of the heart.

As for hypokalemia, except impacts on other functions and systems, heart failure and cardiac rhythm are typical of cardiac symptoms. On the ECG, low, flat, or inverted T-waves and prolongation of the QT interval can be seen. Supraventricular and ventricular extrasystoles occur episodically.

8.5.2. Calcium and acid-base balance

Similarly as the proton is exchanged for the potassium cation, a calcium cation is also exchanged for protons. Plasma proteins play a key role in this mechanism. Blood plasma proteins behave as buffers, primarily due to carboxyl groups and amino groups. As regard the carboxyl groups, these groups are in protonic, nondissociated state (-COOH) in the acidic environment. In the alkaline environment, they begin to buffer and their dissociation into the carboxylate $-COO^-$ occurs, which is able to bind very effective especially Ca^{2+}. It means that in the case of acidosis, the -COOH does not change to $-COO^-$, and in the case of alkalosis, it dissociates to $-COO^-$ and H^+ and the calcium binds to $-COO^-$.

It can also be said that the pH depends on what part of the calcium will be ionized and what part will be nonionized. The practical consequence is that alkalosis leads to ionized hypocalcemia, acidosis, on the contrary, to ionized hypercalcemia. Although total calcium does not change, we have to realize that ionized calcium is metabolically active, especially when it comes to membrane processes.

Hypercalcemia is a state when the serum Ca^{2+} level is greater than 2.8 mmol/l and ionized Ca^{2+} is greater than 1.4 mmol/l. At values above 4 mmol/l, "chemical death" may occur when cardiac arrest may occur. Hypertension and arrhythmias occur at the hypercalcemia. On the ECG, QT interval is shortened. Hypocalcemia is accompanied by an increase in neuromuscular excitability, but myocardial contractility decreases.

8.5.3. Chlorides and acid-base balance

During Cl^- loss (e.g., vomiting), the concentration of the other major ions is not altered, and for maintenance of the electrical neutrality, the anion deficiency is supplemented by an increase in the bicarbonate concentration. pCO_2 does not change; therefore, ventilation is maintained and hypochloremic alkalosis develops. In summary, substitution of chlorides in the blood occurs at the expense of hydrogen carbonates.

9. Conclusions

After summarizing the results from the analysis of acid-base balance parameters (**Table 6**), we concluded that there are differences in the final status of the rat internal environment that depend on the LD cycle and on the type of anesthesia.

In the light part of the day, under P anesthesia, the rats are in a state of acidosis, hypercapnia, and hypoxia, and elevated levels of bicarbonate have been reported. Similarly, it is also in the dark, but with mild acidosis, hypercapnia and hypoxia with a moderate decrease to normal pO_2 values but with elevated levels of bicarbonate. Saturation of hemoglobin by oxygen was at the same level in both light parts of the rat regimen day, and at approximately 87%, the efficiency of the buffer system was not impaired because the values were within the normal range.

Under K/X anesthesia, we found a dependence on LD cycle in all monitored parameters. In the light part of the day, unambiguous acidosis, pCO_2 ranging from normocapnia to hypercapnia, pO in the hypoxic range, relatively large range of bicarbonate (from reduced to increased levels) levels and lower saturation (around 85%) were observed. In the dark part of the day, from normal to alkaline pH, hypocapnia, moderate decreased to normal pO_2 but with a reduced level of bicarbonate. Different values were in saturation of hemoglobin by oxygen, where higher saturation was during the dark (active) part (around 90%). The efficiency of the buffer system moved within the normal range in both light parts of the day.

Under Z anesthesia, the status was as follows: acidosis, hypercapnia, hypoxia to normoxia, and normal levels of bicarbonate in the light part of the day. In the dark part of the day, the state of the internal environment was from acidic to normal, hypercapnia, and pO_2 moved from mild hypoxia to normoxia at a normal to moderately elevated level of bicarbonate. The saturation of hemoglobin by oxygen fluctuated around 89% in both light parts of the rat regimen day, and BB and BE were also in the normal range; thus, buffering capacity remained intact.

It was concluded that P anesthesia is not the most appropriate type of general anesthesia to use in chronobiological rat models. It is likely to produce a more acidic environment than K/X and Z anesthesias, and although an LD difference in P anesthesia was not recorded, the pH values

were the lowest in both light parts of the rat regimen day compared with K/X and Z anesthesias. Initially, acidosis is induced, irrespective of the synchronization of animals with the LD cycle, and therefore, it is not possible to monitor periodic changes in the functions of individual systems that are primarily dependent on changes in extracellular pH. As a result, P probably and immediately reduces either the activity of the buffer systems or inhibits the regulatory mechanisms associated with the maintenance of isohydria, independently of the LD cycle. In this regard, K/X and Z anesthesias may be more appropriate for general anesthesia because the arterial pH varies within the range of isohydria. This assumption is only valid if the rat experiments are performed under K/X and Z anesthesia in the dark (i.e., active) parts of the day.

Hypoxia modifies circadian oscillations of important variables, such as body temperature and metabolism, and may lead to the expectation that the rhythms of many functions are disrupted by hypoxia according to their relationships and association with the primary variables [146]. This effect appears to be apparent in rats under P anesthesia. From a chronobiological point of view, P anesthesia, therefore, is not a suitable form of general anesthesia. Using this type of anesthesia, with the exception of the initial hypoxia and hypercapnia, the LD differences in pO_2 and pCO_2 are eliminated. As a result, the effect of initial hypoxia and hypercapnia on the circadian rhythms of oxygen-dependent systems, immediately after administration of anesthetics, can significantly affect the end result.

Based on the results of this study, we concluded that general anesthesia affects the circadian fluctuation of arterial acid-base balance and plasma concentrations of some ions (**Table 9**). This should be taken into account, and experiments should start with a normal range of acid-base balance. Even at the beginning of the experiment, the altered internal environment may affect the activity of systems whose functions are primarily dependent on acid-base balance.

Anesthetic	Status
Pentobarbital	
Light	Acidosis, from hypoxia to hypercapnia, increased HCO_3, hypernatremia, hyperkalemia, hypocalcemia, hypochloremia
Dark	Acidosis, from normoxia to hypoxia, hypercapnia, increased HCO_3^-, hypernatremia, from normokalemia to hyperkalemia, hypocalcemia, from hypochloremia to normochloremia
Ketamine/xylazine	
Light	Acidosis, hypoxia, from normocapnia to hypercapnia, from decreased to increased level of HCO_3^-, from hyponatremia to hypernatremia, hyperkalemia, hypocalcemia, from normochloremia to hyperchloremia
Dark	From normal pH to alkalosis, from hypoxia to normoxia, hypocapnia, decreased HCO_3^-, from hyponatremia to normonatremia, hyperkalemia, hypocalcemia,
Zoletil	
Light	Acidosis, from hypoxia to normoxia, hypercapnia, normal HCO_3^-, from hyponatremia to hypernatremia, from hypokalemia to hyperkalemia, hypocalcemia, from hypochloremia to hyperchloremia
Dark	From acidosis to normal pH, from hypoxia to normoxia, hypercapnia, from normal to increased HCO_3, from normonatremia to hypernatremia, hyperkalemia, hypocalcaemia, hyperchloremia

Table 9. Internal environment under general anesthesia dependent on the light-dark cycle in the rat.

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References

[1] Subramanian RK, Sidharthan A, Maneksh D, Ramalingam L, Soosai Manickam A, Kanthakumar P, Subramani S. Normative data for arterial blood gas and electrolytes in anesthetized rats. Indian Journal of Pharmacology. 2013;**45**:103-104. DOI: 10.4103/0253-7613.106451

[2] Popoiu CM, Burian CA, Paunescu V, Boia E, Arghirescu S, Muntean DM, Ordodi VL. Development of a high-performance anesthesia ventilator for research in small animals. International Journal of Artificial Organs. 2014;**37**:436-441. DOI: 10.5301/ijao.5000330

[3] Popilskis SJ, Lee DR, Elmore BD. Chapter 12 – Anesthesia and analgesia in nonhuman primates. In: Fish RE, Brown MJ, Danneman PJ, Karas AZ, editors. Anesthesia and Analgesia in Laboratory Animals. 2nd ed. Elsevier Inc: Academic Press; 2008. pp. 335-363

[4] Bruguerolle B, Mesdjan E, Valli M, Blanc MC, Jadot G, Vignon E, Bouyard P. Chronopharmacology of althesin in the adult male rat. Journal de Pharmacologie. 1978;**9**:53-64

[5] Matthews JH, Marte E, Halberg F. A circadian susceptibility-resistance cycle to fluothane in male B1 mice. Canadian Anesthesia Society Journal. 1964;**11**:280-290. https://doi.org/10.1007/BF03002209

[6] Bruguerolle B, Prat M, Douylliez C, Dorfman P. Are there circadian and circannual variations in acute toxicity of phenobarbital in mice? Journal of Fundamental Clinics of Pharmacology. 1988;**2**:301-304. DOI: 10.1111/j.1472-8206.1988.tb00642.x

[7] Yamada Y. Physiology in relation to anesthesia practice: Preface and comments. Japan Journal of Anesthesiology. 2016;**65**:440-441. PMID:27319087

[8] Fijorek K, Puskulluoglu M, Polak S: Circadian Models of serum potassium, sodium, and calcium concentrations in healthy individuals and their application to cardiac electrophysiology simulations at individual level. Computational and Mathematical Methods in Medicine. 2013;**2013**:ID 429037. http://dx.doi.org/10.1155/2013/429037

[9] Stoelting RK, Miller RD: Basic of Anesthesia. 4th edition, Churchill Livingstone; 2000; p. 229. DOI:10.1097/00000539-200105000-00066

[10] Van Slyke DD, Austin JH, Cullen GE. The effects of ether anesthesia on the acid-base balance of the blood. The Journal of Biological Chemistry. 1922;**53**:277-292. http://www.jbc.org/content/53/2/277.citation

[11] Taylor FH, Roos A. Disturbances in acid-base balance during ether anesthesia; with special reference to the changes occurring during thoracic surgery. The Journal of Thoracic Surgery. 1950;**20**:289-295. PMID:ll

[12] Beecher HK, Murphy AJ. Acidosis during thoracic surgery. The Journal of Thoracic Surgery. 1950;**19**:50-70. PMID:15400169

[13] Lucas BGB, Milne EH. Acid base balance and anaesthesia. Thorax. 1955;**10**:354-358. PMC1019436

[14] Payne JP. Acid-base balance in anaesthesia. Anaesthesiall. 2007;**17**:79-80. http://onlinelibrary.wiley.com/doi/10.1111/j.1365-2044.1962.tb13859.x/pdf

[15] Lak M, Araghizadeh H: Acid-base disturbances and related factors in patients under general anesthesia. Iranian Red Crescent Med Journal IRCMJ. 2009;**11**:28-31. ircmj.com/?page=citation_report&article_id=157

[16] Lak M, Araghizadeh H, Fazel K. Acid-base disturbances and its related factors in patients under total intravenous anesthesia. Kowsar Medical Journal. 2011;**15**:221-225. en.journals.sid.ir/JournalList.aspx?id=339

[17] Haskins SC, Patz JD, Farver TD. Xylazine and ketamine-xylazine in dogs. American Journal of Veterinary Research. 1986;**47**:636-641. PMID:3963565

[18] Farver TB, Haskins SC, Patz JD. Cardiopulmonary effects of acepromazine and of the subsequent administration of ketamine in the dog. American Journal of Veterinary Research. 1986;**47**:631-635. PMID:3963564

[19] Stephenson R, Liao KS, Hamrahi H, Horner RL. Circadian rhythms and sleep have additive effects on respiration in the rat. The Journal of Physiology. 2001;**536**(Pt 1):225-235. DOI: 10.1111/j.1469-7793.2001.00225.x

[20] Mortola JP. Breathing around the clock: An overview of the circadian pattern of respiration. European Journal of Applied Physiology. 2004;**91**:119-129. DOI: 10.1007/s00421-003-0978-0

[21] Van Den Buuse M. Circadian rhythms of blood pressure, heart rate, and locomotor activity in spontaneously hypertensive rats as measured with radio-telemetry. Physiology & Behavior. 1994;**55**:783-787. PMID:8190809

[22] Janssen BJA, Tyssen CM, Duindam H, Rietveld WJ. Suprachiasmatic lesions eliminate 24-h blood pressure variability in rats. Physiology & Behavior. 1994;**55**:307-311. https://doi.org/10.1016/0031-9384(94)90138-4

[23] Makino M, Hayashi H, Takezawa H, Hirai M, Saito H, Ebihara S. Circadian rhythms of cardiovascular functions are modulated by the baroreflex and the autonomic nervous system in the rat. Circulation. 1997;**96**:1667-1674. PMID: 9315563

[24] Witte K, Hu K, Swiatek J, Mussing C, Ertl G, Lemmer B. Experimental heart failure in rats: Effects on cardiovascular circadian rhythms and on myocardial β-adrenergic signaling. Cardiovascular Research. 2000;**47**:350-358. PMID: 10946071

[25] Svorc P, Podlubny I, Kujanik S, Bracokova I. 24h rhythm of the vetricular fibrillation threshold during normal and hypoventilation in female Wistar rats. Chronobiology International. 1997;**14**:363-370. PMID: 9262872

[26] Latenkov VP. Diurnal rhythm of acid-base equilibrium and blood gas composition. Bulletin of Experimental Biology and Medicine. 1986;**101**:614-616. PMID: 3708150

[27] Challet E, Malan A, Turek FW, Van Reeth O. Daily variations of blood glucose, acid-base state and PCO2 in rats: Effect of light exposure. Neuroscience Letters. 2004;**355**:131-135. https://doi.org/10.1016/j.neulet.2003.10.041

[28] Pohl HR, Wheeler JS, Murray HE. Chapter 2. Sodium and Potassium in Health and Disease. In: Astrid Sigel, Helmut Sigel, Sigel RKO. Interrelations between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences. 13. Springer; 2013. pp. 29-47. DOI: 10.1007/978-94-007-7500-8_2

[29] Clausen MJV, Poulsen H: Chapter 3 Sodium/Potassium Homeostasis in the Cell. In: Banci Lucia editor. Metallomics and the Cell. Metal Ions in Life Sciences. 12. Springer; 2013. DOI: 10.1007/978-94-007-5561-1_3

[30] Bernardi M, DePalma R, Trevisani F. Serum potassium circadian rhythm. Relationship with aldosterone. Hormone and Metabolic Research. 1985;**17**:695. DOI: 10.1055/s-2007-1013649

[31] Rabinowitz L. Aldosterone and potassium homeostasis. Kidney International. 1996;**49**: 1738-1742. PMID: 8743488

[32] Skotnicka E, Skrzypczak WF, Ozoo M. Circadian changes in electrolyte concentrations in plasma and erythrocytes in two-week-old calves. Acta Veterinaria. 1997;**66**:141-146. https://doi.org/10.2754/avb199766030141

[33] Sennels HP, Jørgensen HL, Goetze JP, Fahrenkrug J. Rhythmic 24-hour variations of frequently used clinical biochemical parameters in healthy young males–the Bispebjerg study of diurnal variations. Scandinavian Journal of Clinical and Laboratory Investigation. 2012;**72**:287-295. DOI: 10.3109/00365513.2012.662281

[34] Sothern RB, Vesely DL, Kanabrocki EL, Bremner FW, Third JLHC, McCormick JB, Dawson S, Ryan M,· Greco J, Bean JT, Nemchausky BM, Shirazi P, Scheving LE: Circadian relationships between circulating atrial natriuretic peptides and serum sodium and chloride in healthy humans. American Journal of Nephrology. 1996;**16**:462-470. DOI: 10.1159/000169045

[35] Nikolaeva S, Pradervand S, Centeno G, Zavadova V, Tokonami N, Maillard M, Bonny O, Firsov D. The circadian clock modulates renal sodium handling. Journal of American Society Nephrology. 2012;**23**:1019-1026. DOI: 10.1681/ASN.2011080842

[36] Moore-Ede MC, Meguid MM, Fitzpatrick GF, Boyden CM, Ball MR. Circadian variation in response to potassium infusion. Clinical Pharmacology and Therapeutics. 1978;**23**: 218-227. DOI: 10.1002/cpt1978232218

[37] Rabinowitz L, Berlin R, Yamauchi H. Plasma potassium and diurnal cyclic potassium excretion in the rat. The American Journal of Physiology. 1987;**253**:F1178-F1181. PMID: 3425726

[38] Aizman RI, Rabinowitz L, Mayer-Harnisch C. Early effects of uninephrectomy on K homeostasis in unanesthetized rats. The American Journal of Physiology. 1996;**270**:R434-R442. PMID: 8779876

[39] Moore Ede MC, Brennan MF, Ball MR. Circadian variation of intercompartmental potassium fluxes in man. Journal of Applied Physiology. 1975;**38**:163-170. PMID: 1110234

[40] Hishida A, Honda N, Kumagai H, Sudo M, Nagase M. Diurnal variation of urinary potassium excretion in chronic renal insufficiency. Mineral and Electrolyte Metabolism. 1982;**7**:20-27. PMID: 6763141

[41] Viana PCR, Caldas-Bussiere MC, Marins RSQS, Menna-Barreto LS, Cury LJ. Diurnal rhythms of plasma ions and metabolites levels in thoroughbred racehorses. Biological Rhythm Research. 2003;**34**:347-354. http://www.tandfonline.com/doi/abs/10.1076/brhm.34.4.347.26230

[42] Salhi A, Centeno G, Firsov D, Crambert G. Circadian expression of H,K-ATPase type 2 contributes to the stability of plasma K^+ levels. FASEB Journal. 2012;**26**:2859-2867. DOI: 10.1096/fj.11-199711

[43] Soták M, Polidarová L, Musílková J, Hock M, Sumová A, Pácha J. Circadian regulation of electrolyte absorption in the rat colon. American Journal of Physiology – Gastrointestinal Liver Physiology. 2011;**301**:G1066-G1074. DOI: 10.1152/ajpgi.00256.2011

[44] Doi M, Takahashi Y, Komatsu R, Yamazaki F, Yamada H, Haraguchi S, Emoto N, Okuno Y, Tsujimoto G, Kanematsu A, Ogawa O, Todo T, Tsutsui K, van der Horst GT, Okamura H: Salt-sensitive hypertension in circadian clock-deficient cry-null mice involves dysregulated adrenal Hsd3b6. Nature Medicine 2010;**16**:67-74. DOI:10.1038/nm.2061

[45] Saifur RM, Emoto N, Nonaka H, Okura R, Nishimura M, Yagita K, van der Horst GT, Matsuo M, Okamura H, Yokoyama M: Circadian clock genes directly regulate expression of the Na(+)/H(+) exchanger NHE3 in the kidney. Kidney International. 2005;**67**: 1410-1419. DOI:10.1111/j.1523-1755.2005.00218.x

[46] Gumz ML, Stow LR, Lynch IJ, Greenlee MM, Rudin A, Cain BD, Weaver DR, Wingo CS. The circadian clock protein period 1 regulates expression of the renal epithelial sodium channel in mice. The Journal of Clinical Investigation. 2009;**119**:2423-2434. DOI: 10.1172/JCI36908

[47] Roelfsema F, Van der Heide D, Smeenk D. Circadian rhythms of urinary electrolyte excretion in freely moving rats. Life Sciences. 1980;**27**:2303-2309. PMID: 7207019

[48] Ball S: Hyponatremia. De Groot LJ, Beck-Peccoz P, Chrousos G, Dungan K, Grossman A, Hershman JM, Koch C, McLachlan R, New M, Rebar R, Singer F, Vinik A, Weickert MO editors: Endotext [Internet] South Dartmouth (MA): MDText.com, Inc. 2000. PMID: 25905359

[49] Williams DM, Gallagher M, Handley J, Stephens JW. The clinical management of hyponatraemia. Postgraduate Medical Journal. 2016;**92**:407-411. DOI: 10.1136/postgradmedj-2015-133740

[50] Henry DA. The clinic: Hyponatremia. Annals of Internal Medicine. 2015;**163**:1-19. DOI: 10.7326/aitc 201508040

[51] Reynolds RM, Padfield PL, Seckl JR: Disorders of sodium balance. British Medical Journal. 2006;**332**:702l705. DOI: 10.1136/bmj.332.7543.702

[52] Lin M, Liu SJ, Lim IT. Disorders of water imbalance. Emergency Medicine Clinics of North America. 2005;**23**:749-770. DOI: 10.1016/j.emc.2005.03.001

[53] Brini M, Ottolini D, Calì T, Carafoli E: Chapter 4. Calcium in Health and Disease". In Sigel A, Sigel H, Sigel RKO, editors. Interrelations between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences. 13. Spring; 2013a. p. 81-137. DOI: 10.1007/978-94-007-7500-8_4

[54] Brini M, Cali T, Ottolini D, Carafoli E: Chapter 5 Intracellular Calcium Homeostasis and Signaling. In Banci Lucia editor. Metallomics and the Cell. Metal Ions in Life Sciences. 12. Springer; 2013b. DOI:10.1007/978-94-007-5561-1_5

[55] Minisola S, Pepe J, Piemonte S, Cipriani C. The diagnosis and management of hypercalcaemia. BMJ. 2015;**350**:h2723. DOI:10.1136/bmj.h2723

[56] Fong J, Khan A: Hypocalcemia: Updates in diagnosis and management for primary care. Canadian Family Physician Medecin de Famille Canadien. 2012;**58**:158-162. PMID: 22439169

[57] Strichartz G Molecular mechanisms of nerve block by local anesthetics. Anesthesiology 1976;**45**:421-441. PMID: 9844

[58] Ko GYP, Shi L, Ko MLJ. Circadian regulation of ion channels and their functions. Neurochemistry. 2009;**110**:1150. DOI: 10.1111/j.1471-4159.2009.06223.x

[59] Reinberg A. Circadian rhythms in effects of hypnotics and sleep inducers. International Journal of Clinical Pharmacology Research. 1986;**6**:33-44. PMID: 3514493

[60] Sato Y, Seo N, Kobahashi E. The dosing-time dependent effects of intravenous hypnotics in mice. Anesthesia and Analgesia. 2005;**101**:1706-1708. DOI: 10.1213/01.ANE.0000184127.67866.2E

[61] De Oliveira RB, De Macedo DV, Santos GB, Arcanjo AM: Reliability of the electrocardiogram in normal rats. International Journal of Exercise Science. 2012; Conference Proceedings 1: Article 63. http://digitalcommons.wku.edu/cgi/viewcontent.cgi?article=1449&context=ijesab

[62] Lewis LD, Ponten U, Siesjo BK. Arterial acid-base changes in un-anesthetized rats in acute hypoxia. Respiration Physiology. 1974;**19**:312-321. DOI: 10.1016/0034-5687(73)90035-2

[63] Pepelko WE, Dixon GA. Arterial blood gases in conscious rats exposed to hypoxia, hypercapnia, or both. Journal of Applied Physiology. 1975;**38**:581-587. PMID: 237863

[64] Brun-Pascaud M, Gaudebout C, Blayo MC, Pocidalo JJ. Arterial blood gases and acid-base status in awake rats. Respiration Physiology. 1982;**48**:45-57. PMID: 6810430

[65] Girard P, Brun-Pascaud M, Pocidalo JJ. Acid-base status of awake rats after cannulation of aorta and vena cava. Kidney International. 1983;**24**:795-799. https://doi.org/10.1038/ki.1983.230

[66] Hess L, Dvořáček I, Svobodník J: Chapter 3, Rats (Rodentia). In: Anesthesia of laboratory animals. Prague: Avicenum; 1988. p. 158

[67] Dettmers CH, Hagendorff A, Kastrup A, Luderitz B, Hartmann A. An experimental model for hemodynamic evaluation of arrhythmias in rats. Cerebrovascular Diseases. 1994;**4**:309-313. DOI: 10.1159/000108500

[68] Chi OZ, Wei HM, Tse J, Klein SL, Weiss HR. Cerebral microregional oxygen balance during chronic versus acute hypertension in middle cerebral artery occluded rats. Anesthesia and Analgesia. 1996;**82**:587-592. PMID: 8623966

[69] Ohoi I, Takeo S. Involvement of superoxide and nitric oxide in the genesis of reperfusion arrhythmias in rats. European Journal of Pharmacology. 1996;**306**:123-131. https://doi.org/10.1016/0014-2999(96)00231-2

[70] Schultz JJ, Hsu AK, Gross GJ. Ischemic preconditioning and morphine-induced cardioprotection involve the delta (δ)-opioid receptor in the intact rat heart. Journal of Molecular and Cellular Cardiology. 1997;**29**:2187-2195. DOI: 10.1006/jmcc.1997.0454

[71] Sun W, Wainwright CL. The role of nitric oxide in modulating ischaemia-induced arrythmias in rats. Journal of Cardiovascular Pharmacology. 1997;**29**:554-562. PMID:9156367

[72] Forkel J, Chen X, Wandinger S, Keser F, Duschin A, Schwanke U, Frede S, Massoudy P, Schulz R, Jakob H, Heusch G. Responses of chronically hypoxic rat hearts to ischemia: K_{ATP} channel blockade does not abolish increased RV tolerance to ischemia. American Journal of Physiology. Heart and Circulatory Physiology. 2004;**286**:H545-H551. DOI: 10.1152/ajpheart.00022.2003

[73] Valenza F, Pizzocri M, Salice V, Chevallard G, Fossali T, Coppola S, Froio S, Polli F, Gatti S, Fortunato F, Comi GP, Gattinoni L. Sodium bicarbonate treatment during transient or sustained lactic acidemia in normoxic and normotensive rats. PLoS One. 2012;**7**:e46035. DOI: 10.1371/journal.pone.0046035

[74] Peralta-Ramírez A, Raya AI, Pineda C, Rodríguez M, Aguilera-Tejero E, López I. Acid-base balance in uremic bats with vascular calcification. Nephron Extra. 2014;**4**:89-94. DOI: 10.1159/000363298

[75] Luo X, Yin Y, You G, Chen G, Wang Y, Zhao J, Wang B, Zhao L, Zhou H. Gradually increased oxygen administrativ improved oxygenation and mitigated oxidative stress after resuscitation from severe hemorrhagic shock. Anesthesiology. 2015;**123**:1122-1132. DOI: 10.1097/ALN.0000000000000859

[76] Menegon LF, Figueiredo JF, Gontijo JAR. Effect of metabolic acidosis on renal tubular sodium handling in rats as determined by lithium clearance. Brazilian Journal of Medical and Biological Research. 1998;**31**:1269-1273. DOI: 10.1590/S0100-879X1998001000006

[77] Da Silva Costa ECD, Goncalves AA, Areas MA, Morgabel RGB. Effects of metformin on QT and QTc interval dispersion of diabetic rats. ARQUIVOS BRASILEIROS DE CARDIOLOGIA. 2008;**90**:254-260. DOI: 10.1590/S0066-782X2008000400004

[78] Dispersyn G, Pain L, Challete E, Touitou Y. General anesthetics effects on circadian temporal structure: An update. Chronobiology International. 2008;**25**:835-850. http://dx.doi.org/10.1080/07420520802551386

[79] Ebihara S, Goto M, Oshima I. The phase-shifting effects of pentobarbital on the circadian rhythm of locomotor activity in the mouse: Strain differences. Brain Research. 1988;**454**:404-407. https://doi.org/10.1016/0006-8993(88)90847-5

[80] Ebihara S, Hayakawa H. Pentobarbital-induced phase shifts of circadian rhythms of locomotor activity are not mediated through stimulated activity in mice. Experientia. 1990;**46**:1023-1026. PMID: 2226716

[81] Pang CS, Mulnier C, Pang SF, Yang JC. Effects of halothane, pentobarbital and ketamine on serum melatonin levels in the early scotophase in New Zealand white rabbits. Biological Signals and Receptors. 2001;**10**:310-316. DOI: 10.1159/000046898

[82] Ohno R, Miyata H, Kimura M. Circadian rhythms and effects of anesthesia on plasma natriuretic peptide levels in rats. Yakugaku Zasshi. 2009;**129**:1529-1535. PMID: 19952533

[83] Naguib M, Gottumukkala V, Goldstein PA. 2007. Melatonin and anesthesia: A clinical perspective. Journal of Pineal Research. 2007;**42**:12-21. DOI: 10.1111/j.1600-079X.2006.00384.x

[84] Reber A, Huber PR, Ummenhofer W, Gürtler CM, Zurschmiede C, Drewe J, Schneider M. General anesthesia for surgery can affect circulating melatoninduring daylight hours. Acta Anaesthesiologica Scandinavica. 1998;**42**:1050-1056. PMID: 9809087

[85] Nishimura S, Fujino Y, Shimaoka M, Hagihira S, Taenaka N, Yoshiya I. Circadian secretion patterns of melatonin after major surgery. Journal of Pineal Research. 1998;**25**:73-77. PMID: 9755027

[86] Kärkelä J, Vakkuri O, Kaukinen S, Huang WQ, Pasanen M. The influence of anaesthesia and surgery on the circadian rhythm of melatonin. Acta Anaesthesiologica Scandinavica. 2002;**46**:30-36. PMID: 11903069

[87] Coloma FM, Niles LP. Melatonin enhancement of [3 H] -c-aminobutyric acid and [3 H] muscimol binding in rat brain. Biochemical Pharmacology. 1988;**37**:1271-1274. https://doi.org/10.1016/0006-2952(88)90781-2

[88] Niles LP, Pickering DS, Arciszewski MA. Effects of chronic melatonin administration on GABA and diazepambinding in rat brain. Journal of Neural Transmission. 1987;**70**:117-124. PMID: 3668517

[89] Wang F, Li J, Wu C, Yang J, Xu F, Zhao Q. The GABA(a) receptor mediates the hypnotic activity of melatonin in rats. Pharmacology, Biochemistry, and Behavior. 2003;**74**:573-578. PMID: 1254322

[90] Mihara T, Kikuchi T, Kamiya Y, Koga M, Uchimoto K, Kurahashi K, Goto T. Day or night administration of ketamine and pentobarbital differentially affect circadian rhythms of pineal melatonin secretion and locomotor activity in rats. Anesthesia and Analgesia. 2012;**115**:805-813. DOI: 10.1213/ANE.0b013e3182632bcb

[91] Dispersyn G, Pain L, Touitou Y. Propofol Anesthesia significantly alters plasma blood levels of melatonin in rats. Anesthesiology. 2010;**112**:333-337. DOI: 10.1097/ALN.0b013e3181c920e2

[92] Kana K, Iijima N, Ohe Y, Ozawa H, Sakamoto A. Effect of the general anesthetic sevoflurane on *mPer2* expression and behavioral rhythm is dependent on the time in a day. Anesthesiology. 2011. Annual meeting, abstract A1007, October 17

[93] Prudian F, Gantenbein M, Pelissier AL, Attolini L, Bruguerolle B. (1997). Daily rhythms of heart rate, temperature and locomotor activity are modified by anaesthetics in rats: A telemetric study. Naunyn–Schmiedebergs Archives of Pharmacology. 1997;**355**:774-778. PMID: 9205963

[94] Pelissier AL, Gantenbein M, Prudian F, Bruguerolle B. Influence of general anaethetics on circadian rhythms of heart rate, body temperature and locomotor activity in rats. Sciences et Techniques de l Animal de Laboratoire. 1998;**23**:91-98

[95] Gerst PH, Fleming WH, Malm JR. A quantitative evaluation of the effects of acidosis and alkalosis upon the ventricular fibrillation threshold. Surgery. 1966;**59**:1050-1066. PMID: 5937951

[96] Rogers RM, Spear JF, Moore EN, Horowitz LH, Sonne JE. Vulnerability of canine ventricle to fibrillation during hypoxia and respiratory acidosis. Chest. 1973;**63**:986-994. https://doi.org/10.1378/chest.63.6.986

[97] Ferrier GR, Moffat MP, Lukas A. Possible mechanisms of ventricular arrhythmias elicited by ischemia followed by reperfusion. Circulation Research. 1985;**56**:184-194. http://circres.ahajournals.org/content/circresaha/56/2/184.full.pdf

[98] Nishimura M, Tanaka H, Homma N, Matsuzawa T, Watanabe Y. Ionic mechanisms of the depression of automaticity and conduction in the rabbit atrioventricular node caused by hypoxia or metabolic inhibition and protective action of glucose and valine. American Journal of Cardiology. 1989;**64**:24J-28J. PMID:2480705

[99] Nisbet AM, Burton FL, Walker NL, Craig MA, Cheng H, Hancox JC, Orchard CH, Smith GL. Acidosis slows electrical conduction through the atrio-ventricular node. Frontiers in Physiology. 2014;**5**:233. DOI: 10.3389/fphys.2014.00233

[100] Svorc P, Bacova I, Gresova S, Svorc P Jr. Chronobiological perspectives on myocardial electrophysiological parameters under th4ree types of general anaesthesia in a rat model. Biological Rhythm Research. 2017;**48**:343-351. http://dx.doi.org/10.1080/09291016.2016.1263003

[101] Kaczmarczyk G, Reinhardt HW. Arterial blood gas tensions and acid-base status of wistar rats during thiopental and halothane anesthesia. Laboratory Animal Science. 1975;**25**:184-190. PMID:237143

[102] Komarek J. The effect of ether, pentobarbitone sodium and fentanyl on blood gases, acid-base balance and hematological parameters in the rat. Experientia. 1984;**40**:476-478. PMID: 6426998

[103] Alfaro V, Palacios L. Differential effects of hypothermia upon blood acid-base state and blood gases in sodium pentobarbital and urethane anaesthetised rats. General Pharmacology. 1992;**23**:677-682. https://doi.org/10.1016/0306-3623(92)90147-C

[104] Alfaro V, Palacios L. Comparison of acid-base status in conscious and anesthetized rats during acute hypothermia. Pflugers Archiv-European Journal of Physiology. 1993;**424**:416-422. DOI: 10.1007/BF00374903

[105] Gaudy JH, Sicard JF, Maneglia R, Atos MQ. Effects of halothane on $paCO_2$ variability, acid-base-balance and ventilation induced hypoxia in the rat. Canadian Journal of Anaesthesia. 1994;**41**:347-352. https://doi.org/10.1007/BF03009916

[106] Alfaro V, Palacios L. Components of the blood acid-base disturbance that accompanies urethane anaesthesia in rats during normothermia and hypothermia. Clinical and Experimental Pharmacology & Physiology. 1997;**24**:498-502. DOI: 10.1111/j.1440-1681.1997.tb01234.x

[107] Buelke-Sam J, Holson JF, Bazare JJ, Young JF. Comparative stability of physiological parameters during sustained anesthesia in rats. Laboratory Animal Science. 1978;**28**: 157-162. PMID: 642434

[108] Akça O, Doufas AG, Morioka N, Iscoe S, Fisher J, Sessler DI. Hypercapnia improves tissue oxygenation. Anesthesiology. 2002;**97**:801-806 PMID:12357143

[109] Ohshima Y, Iwase M, Izumizaki M, Ishiguro T, Kanamaru M, Nakayama H, Gejyo F, Homma I. Hypoxic ventilatory response during light and dark periods and the involvement of histamine H1 receptor in mice. American Journal of Physiology. Regulatory, Integrative and Comparative Physiology. 2007;**293**:R1350-R1356. DOI: 10.1152/ajpregu.00318.2007

[110] Iwase M, Ohshima Y, Izumizaki M, Homma I. Circadian changes in respiratory responses to acute hypoxia and histamine H1 receptors in mice. Advances in Experimental Medicine and Biology. 2010;**669**:235-238. DOI: 10.1007/978-1-4419-5692-7_47

[111] Lenhardt R. The effect of anesthesia on body temperature control. Frontiers in Bioscience (Scholar Edition). 2010;**2**:1145-1154. PMID: 20515846

[112] Díaz M, Becker DE. Thermoregulation: Physiological and clinical considerations during sedation and general anesthesia. Anesthesia Progress. 2010;**57**:25-33. DOI: 10.2344/0003-3006-57.1.25

[113] Torbati D, Camacho MT, Hultquist K, Sussmane JB, Raszynski A, Wolfsdorf J: Ventilation and gas-exchange responses to hypothermia and pentobarbital anesthesia in female rats. Critical Care Medicine. 1998;**26**(1 suppl):A119. journals.lww.com › Home › January 1998 - Volume 26 - Issue 1

[114] Torbati D, Camacho MT, Raszynski A, Sussmane JB, Totapally BR, Hultquist K, Wolfsdorf J. Effect of hypothermia on ventilation in anesthetized, spontaneously breathing rats: Theoretical implications for mechanical ventilation. Intensive Care Medicine. 2000;**26**:585-591. DOI: 10.1007/s001340051208

[115] Piccione G, Caola G, Mortola JP. Day/night pattern of arterial blood gases in the cow. Respiratory Physiology & Neurobiology. 2004;**140**:33-41. DOI: 10.1016/j.resp.2003.11.008

[116] Pask EA. Artificial ventilation. Anaesthesia. 1948;**3**:58-66

[117] Aletti I, Beatrice E, Rolfo F, Cuzzupoli F, Vidili F. Effect of automatic artificial ventilation on acid-base equilibrium and on hemodynamics in various experimental conditions. Minerva Anestesiologica. 1961;**27**:37-53

[118] Perrin LF, Descottes J, Martin MR, Labry M. Variations of acid-base equilibrium under artificial ventilation. Bulletin de la Société Internationale de Chirurgie. 1961;**20**:165-172

[119] Albers C. Ventilatory control of the acid-base equilibrium in hypothermia. Der Anaesthesist. 1962;**11**:43-51

[120] Fagbeni O, Kane KA, Parratt JR. Creatine phosphate suppress ventricular arrhythmias resulting from coronary artery ligation. Journal of Cardiovascular Pharmacology. 1982;**4**:53-58. DOI: 10.1097/00005344-198201000-00009

[121] Richard V, Tron C, Thuilles C. Ischaemic preconditioning is not mediated by oxygen derived free radicals in rats. Cardiovascular Research. 1993;**27**:2016-2021. PMID: 8287412

[122] Guarini S, Martini MC, Bertolini A. Reperfusion-induced arrhythmias and lethality are reduced by a 2KDa heparin fragment. Life Sciences. 1995;**57**:967-972. PMID: 7643721

[123] Lott FD, Guo P, Toombs CF. Reduction in infarct size by ischemic preconditioning persists in a chronic rat model of myocardial ischemia-reperfusion injury. Pharmacology. 1996;**52**:113-118. https://doi.org/10.1159/000139374

[124] Godin-Ribuot D, Ribuot C, Lamontagne D, Yamaguchi N, Couture R, de Champlain J, Demenge P, Nadeau R: Reflex adrenal medullary secretion during coronary occlusion mediated by cardiac receptors with afferent vagal fibres in the rat. Pfügers Archiv. 1997;**434**:159-165. DOI: 10.1007/s004240050378

[125] Oosting J, Struijker-Boudier HA, Janssen BJ. Circadian and ultradian control of cardiac output in spontaneous hypertension in rats. American Journal of Physiology. 1997;**273**(1,Pt2):H66-H75. PMID: 9249476

[126] Häfner D, Germann PG, Hauschke D. 1998. Effects of rSP-C surfactant on oxygenation and histology in a rat-lung-lavage model of acute lung injury. American Journal of Respiratory and Critical Care Medicine. 1998;**158**:270-278. https://doi.org/10.1164/ajrccm.158.1.9712061

[127] Tanno M, Tsuchida A, Nozawa J, Matsumoto T, Hasegawa T, Miura T, Shimamoto K. Roles of tyrosine kinase and protein kinase C in infarct size limitation by repetitive ischemic preconditioning in the rat. Journal of Cardiovascular Pharmacology. 2000;**35**:345-352. PMID: 10710117

[128] Ravingerova T, Neckař J, Kolař F, Stetka R, Volkovova K, Ziegelhöffer A, Styk J. Ventricular arrhythmias following coronary artery occlusion in rats: Is the diabetic heart less or more sensitive to ischaemia? Basic Research in Cardiology. 2001;**96**:160-168. PMID:11327334

[129] Neckař J, Papoušek F, Nováková O, Oštádal B, Kolář F. Cardioprotective effects of chronic hypoxia and ischaemic preconditioning are not additive. Basic Research in Cardiology. 2002;**97**:161-167. PMID: 12002264

[130] Neckař J, Oštádal B, Kolář F. Acute but not chronic tempol treatment increases ischemic and reperfusion ventricular arrhythmias in open-chest rats. Physiological Research. 2008;**57**:653-656. http://www.biomed.cas.cz/physiolres/pdf/57/57_653.pdf

[131] Fraley DS, Adler S. Isohydric regulation of plasma potassium by bicarbonate in the rat. Kidney International. 1976;**9**:333-343. https://doi.org/10.1038/ki.1976.39

[132] Fenn WO, Cobb DM. The potassium equilibrium in muscle. The Journal of General Physiology. 1934;**17**:629-645. DOI: 10.1085/jgp.17.5.629

[133] Giebisch G, Berger L, Pitts RF. The extrarenal response to acute acid-base disturbances of respiratory origin. The Journal of Clinical Investigation. 1955;**34**:231-245. DOI: 10.1172/JCI103076

[134] Scribner RH, Fremont-Smith K, Burnell JM. The effect of acute respiratory acidosis on the internal equilibrium of potassium. The Journal of Clinical Investigation. 1955;**34**:1276-1285. DOI: 10.1172/JCI103174

[135] Burnell JM, Villamil MF, Uyeno BT, Scribner GH. The effect in humans of extracellular pH change on the relationship between serum potassium concentration and intracellular potassium. The Journal of Clinical Investigation. 1956;**35**:935-939. DOI: 10.1172/JCI103352

[136] Katzman R, Villee CA, Beecher HK. Effect of increased carbon dioxide concentrations on fixed acid production in vitro. The American Journal of Physiology. 1953;**72**:317-323. ajplegacy.physiology.org/content/172/2/317

[137] Longmore WJ, Hastings AB, Mahowald TA. Effect of environmental CO_2 and pH on glycerol metabolism by rat liver in vitro. The Journal of Biological Chemistry. 1964;**239**:1700-1704. http://www.jbc.org/content/239/6/1700.citation

[138] Kamm DE, Fuisz RE, Goodman AD, Cahill GF Jr. Acidbase alterations and renal gluconeogenesis: Effect of pH, bicarbonate concentration, and pCO_2. The Journal of Clinical Investigation. 1967;**46**:1172-1177. DOI: 10.1172/JCI105610

[139] Adler S. The role of pH, pCO_2 and bicarbonate in regulating rat diaphragm citrate content. The Journal of Clinical Investigation. 1970;**49**:1647-1655. DOI: 10.1172/JCI106382

[140] Simmons DH, Avedon M. Acid-base alterations and plasma potassium concentration. The American Journal of Physiology. 1959;**197**:319-326. ajplegacy.physiology.org/content/ajplegacy/197/2/319.full.pdf

[141] Leibman J, Birkenfield LW, Edelman IS. The dependence of plasma potassium concentration on plasma osmolarity, blood pH, and body composition. The Journal of Clinical Investigation. 1958;**37**:9-10

[142] Kim WG, Brown EB Jr. Potassium transfer with constant extracellular pH. The Journal of Laboratory and Clinical Medicine. 1968;**71**:678-685. http://www.translationalres.com/article/0022-2143(68)90082-6/pdf

[143] Makoff DL, Dasilva JA, Rosenbaum BJ. On the mechanism of hyperkalemia due to hyperosmotic expansion with saline or mannitol. Clinical Science. 1971;**41**:383-393. DOI: https://doi.org/10.1042/cs0410383

[144] Komai H, Rusy BF. Effects of bupivacaine and lidocaine on AV conduction in the isolated rat heart: Modification by hyperkalemia. Anesthesiology. 1981;**55**:281-285. PMID: 7270953

[145] Kuwahara M, Chiku K, Shiono T, Tsubone H, Sugano S. ECG changes under hyperkalemia with nephrectomy in the rat. Journal of Electrocardiology. 1992;**25**:215-219. PMID: 1645061

[146] Mortola JP. Hypoxia and circadian patterns. Respiratory Physiology & Neurobiology. 2007;**158**:274-279. DOI: 10.1016/j.resp.2007.02.005

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