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Selected aspects of chronobiological studies in anaesthesia

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Abstract

In humans many physiological processes present diurnal variations controlled by an underlying circadian clock. Both anaesthesia and circadian clock may influence each other. Recent lines of evidence have also suggested that natural sleep and anaesthesia may be more similar than previously realized. Sleep deprivation enhances the potency of anaesthetics, such as propofol, establishing a link between the need for and anaesthesia. Works on rats suggest that during prolonged anaesthesia, sleep need does not accrue and that recovery from sleep deprivation can occur during anaesthesia. Some receptor-based effects of general anaesthetics appear to occur at the tuberomammillary nucleus (TMN) of the hypothalamus, a known sleep regulatory center in the brain. For many drugs used in anaesthesia the time-of-day effect has been confirmed, however mostly on animals. Further studies on humans are required to verify the obtained results. Despite of typical considerations for drug dosing, such as age, gender, weight, the diurnal fluctuations in drug responses may be important. Considering the time-of-day as a covariate for PK/PD (Pharmacokinetics/Pharmacodynamics) modeling of anaesthetic drugs in volunteers as well as in clinical settings the real role of circadian rhythms in various clinical conditions should be proven. Our aim was to review recent advances in our understanding of the relevance of circadian rhythms for anaesthesia as well as the methodological problems and considerations for assessing circadian rhythms in laboratory conditions and clinical settings. *Anestezjologia i Ratownictwo 2014; 8: 38-50.*

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The circadian clock and anaesthesia

The circadian timing system in mammals is thought to be organized in a hierarchical way. The main circadian clock is located in the suprachiasmatic nuclei (SCN) of the anterior hypothalamus. However, many other brain regions and most peripheral tissues also contain circadian clocks with varying levels of dependence on the SCN for sustained rhythmicity [1-3]. Both anaesthesia and circadian clock may influence each other. Some anaesthetic drugs can act directly on the clock itself, shifting it to a different phase. Several studies show clear phase-shifting effects of anaesthetic drugs [4,5]. It has been also demonstrated that general

anaesthesia for surgery influences melatonin and cortisol levels. During the postoperative period an increased level of melatonin with a decreased level of cortisol were simultaneously observed [6]. The effects on melatonin were also confirmed for general anaesthesia per se, since propofol anaesthesia in rats has been shown to phase advance the secretion of melatonin [7] which is known factor controlling circadian rhythms [8]. On the other hand, it has been shown that the circadian clock may affect anaesthesia. It has been established for rodents and rabbits that the efficacy of anaesthesia is better with anaesthetic administration during the rest phase, as compared with the activity phase [3]. Recent works have also demonstrated that exogenously administrated melatonin may be effective in premedication in both adult and surgical patients, whereas when given intravenously, it acts as an anaesthetic agent in the rat model [9-11]. Naguib et al. tested different doses of melatonin as a drug for premedication in adults in a double-blinded placebo controlled study, and discovered that 0.05 mg/kg melatonin caused anxiolysis with no co-existing cognitive, psychomotor dysfunction, nor the quality of recovery [10]. Melatonin was also tested as a premedication in children with a good recovery, less postoperative agitation, and reduced the number of sleep disturbances cases after hospitalization [11]. On the animal experimental model, it was shown that melatonin exerts sleep-promoting potency comparable to this caused by propofol and thiopental. Very likely, it is achieved by allosteric modulation of GABA-A receptor in the suprachiasmatic nuclei [11]. If used before the intravenous regional anaesthesia (IVRA), melatonin not only reduced preoperative anxiety, but also diminished pain developing during the tourniquet application and improved the postoperative pain treatment [12]. Postoperatively, there were cases of delirium occurring after anaesthesia and surgical procedure resistant to the standard treatment and cured with melatonin, as shown by Hanania and Kitain [13].

General anaesthesia and natural sleep

The idea that anaesthesia and sleep may share common neurophysiological elements has been supported by behavioral studies on animals [14-16] as well as by pharmacological studies [17]. The observation that sleep deprivation seems to enhance anaesthetic potency, the separate observation that it is relieved by prolonged anaesthesia [15,18] and the fact that both sleep and anaesthesia induce hypothermia all add weight to the concept that sleep and anaesthesia have some common mechanisms [19]. Some direct evidence comes from the work on specific neuronal pathways. Almost all anaesthetic drugs have been shown to potentiate GABA – induced Cl^- currents, and generally at higher concentrations, directly activate $GABA_A$ receptors in the absence of GABA [19]. Nelson et al. administered GABA antagonist gabazine into the TMN (tuberomammillary nucleus) of rats, which was noted to significantly alter the hypnotic effect of GABAergic anaesthetics (propofol, muscimol and pentobarbital) but not of the non-GABAergic anaesthetic ketamine [17]. These findings indicate that at least one of the mechanisms by which GABA-based anaesthetics produce hypnosis is via GABA-mediated actions on the brain nuclei, which are known to participate in sleep generation. Anaesthetics appear also to act on other brain regions, which are also involved in sleep generation. Direct administration of the anaesthetic propofol into the medial preoptic area of the rat brain increased subsequent sleep in a dose dependent fashion [20]. The cerebral blood flow (CBF) and glucose metabolism are indirect methods of measuring the neuronal activity. Most studies that measured the CBF or glucose metabolism highlighted the importance of thalamic deactivation during anaesthetic-induced LOC (lost of consciousness) [21-23]. Clear similarities between anaesthetic-induced LOC and natural sleep are largely caused by deactivation of the thalamus, which occurs in both conditions, leading to a similar pattern of cortical inhibition [19,24].

Both interactions between the circadian clock and anaesthesia as well as the similarity between natural and drug-induced sleep potentially make circadian rhythms in the pharmacodynamics of anaesthetics possible.

Chronopharmacokinetics and chronopharmacodynamics of anaesthetics

The time of drug administration may influence the response of the organism. Moreover, the different steps in pharmacokinetics, e.g. absorption, distribution, metabolism and elimination, are influenced by different physiological functions that may vary over the 24 hr scale. The pharmacokinetic parameters are conventially considered to be constant in time. Rarely

their circadian time-dependent variations are assessed [25]. Due to large inter-individual variability in the PK and PD parameters of anaesthetics, both the over and underdosage of these drugs still remain a problem in everyday clinical practice. Concerning the results of recent studies, the time-of-day may by an additional factor, which needs to be considered by anesthesiologists.

General anaesthetics

The animal studies have suggested that the maximum hypnotic effect of general anaesthetic occurs during the rest phase, especially for the drugs modulating $GABA_A$ neurotransmission. Most investigators suppose that longer hypnosis during the rest hours is due to circadian rhythms in drug pharmacodynamics rather than pharmacokinetics. However, recent studies provide evidence that pharmacokinetics of hypnotics and sedatives may also be time-dependent [26,27]. The first studies on chronobiology of hypnotics were made on barbiturates by Davis in 1962 and found out that maximum effect is achieved after administration at the rest phase [28]. Naum & Golombek observed that etomidate, a drug that positively modulates $GABA_A$-mediated neurotransmission, induced a significant loss of the righting reflex when when administrated at 12:00 but not at 24.00 in hamsters [29]. On the contrary, ketamine, which induces anaesthesia by inhibiting glutamanergic neurotransmission, did not show a diurnal variation in the loss of the righting reflex test. Opposite results were obtained in the study by Sato et al., who noted the enhanced hypnotic activity of ketamine during the early active phase in mice with no variations in ketamine blood concentrations [30]. The authors concluded the results might be due to daily variations in anaesthetic sensitivity in the CNS. Similarly, Rebuelto et al. observed diurnal changes in the pharmacological response to ketamine [31,32]. The authors found that the longest anaesthetic response to ketamine or a combination of ketamine and midazolam occurred during the day (natural sleep period) in rats. Challet et al. demonstrated that the duration of propofol anaesthesia in rats exhibits a threefold amplitude variation, depending on the time of administration [33]. The longest duration occurred during the natural sleep period (30 min versus 10 min during the activity phase). Because the authors did not measure propofol concentrations in this study it was not possible to discriminate whether the PK and/or PD are responsible for the within-day temporal variation in the degree of anaesthesia. Similar results have recently been obtained in rabbits [26]. The authors observed the smallest sedation effect of propofol during the activity period. In this study the diurnal fluctuations in both pharmacodynamics and pharmacokinetics of propofol were noted, concluding, not only pharmacodynamics of hypnotics may be time-dependent. Sato et al. has also demonstrated the time-of-day-dependent anaesthetic effects for propofol and other hypnotic agents in mice [34]. However, the authors did not observe any circadian variations in the total content of cytochrome P 450 enzymes (CYPs) and activities of isoenzymes in the liver, concluding the dosing time-dependent effect of hypnotics may be explained by differing sensitivity of the CNS rather than pharmacokinetics. The lack of concentration measurements in blood remains the limitation of this study, because for highly extracted drugs the cardiac output (CO) is the major determinant of the rate of the clearance rather than CYP activity. Alternatively to variation in the CNS sensitivity to propofol the night-day differences in rats CO might explain the results obtained by Challet et al., who noted longer propofol hypnosis during the rest period in rats [33]. As far as inhaled anaesthetics are concerned, the potency of halothane has been shown to exhibit temporal changes in rats with the anaesthetic potency (MAC) being lower at 12:00 (1.26%) than at 20:00 (1.45%) (Munson et al., 1970) [35]. There is very little data concerning the chronobiology of newer inhaled hypnotics, i.e. isoflurane, enflurane, desflurane, sevoflurane. Ohe et al. performed an interesting genetic study, in which the authors tested the sensitivity of the molecular clock to the volatile hypnotics. Sevoflurane affected the expression of clock gene mPer2 by 64.5% in a reversible manner accompanying the change of Nicotinamide Adenine Dinecluotide (NAD^+) level in the suprachiasmatic nucleus of mice and yields an important information into the nature of postoperative sleep disturbances [36]. Dexmedetomidine, which uses α2-adrenergic mechanism of action, was also studied in relation to the genetic dysfunction by Yoshida et al. The scientists compared the effect of dexmedetomidine on the genetic system with propofol and 10% intralipid. Hypnotics, but not intralipid, shared the similar depressive activity on the molecular clock genes [37].

Taking into consideration the results obtained in animals, similar studies are required in humans to confirm the temporal changes in the anaesthetic response

and to find the relationships between the time of day and PK/PD. To our knowledge, there are only two studies performed in humans. The older one showed that the maximum efficacy of halothane (as indicated by consumption of the agent) was between 00:00h and 06:00h [38]. Recently Bienert et al. have not observed any circadian changes in propofol PK and PD during prolonged infusion in ICU patients [39]. However, the monitored physiological parameters, like blood pressure, heart rate, blood oxygenation, body temperature did not demonstrate normal, healthy pattern in these patients. The lack of a normal circadian profile in various physiological functions unquestionably influence the possible chronopharmacological profile of the drugs. The results of different studies examining the circadian PK and PD of hypnotics are summarized in table I.

Further studies are required to examine the real role of time of the day in the pharmacology of anaesthetics in healthy subjects as well as in ICU settings. It is especially important in view of the fact that recent studies showed that common methods used to measure the depth of hypnosis, like BIS, are not able to prevent anaesthesia awareness in all patients and they may fail in ICU patients [40-43]. Thus, there is a need to search for and identify the factors influencing the PK and PD of the drugs used to obtain sedation and unconsciousness.

Benzodiazepines

Benzodiazepines act on the $GABA_A$ receptor and have sedative, anxiolytic, muscle-relaxing and amnesic properties. Naranjo et al. studied daily fluctuations in diazepam, N-desmethyldiazepam and diazepam free fraction in volunteers [44]. They found that the within-day total diazepam and N-desmethyldiazepam concentrations varied significantly [$p < .001$], being lower between 23:00 and 08:00 h and higher at 09:00 h. The diazepam free fraction also varied significantly [$p < 0.001$], but it was the highest between 23:00 and 08:00 and lowest at 09:00 h. Short acting benzodiazepines (BZDs), i.e. midazolam, triazolam were found to have phase-shifting effects on the circadian clock of the hamster [45,47]. Also, it has been observed that time of the day may significantly influence the pharmacokinetics of diazepam and midazolam. However, the chronopharmacology of BZDs has not been fully established yet. Recently, Tomalik-Scharte et al. noted circadian variability in the clearance of intravenous midazolam, given to healthy subjects [27]. The authors concluded that liver enzyme activity demonstrates diurnal variations in humans. On the contrary, Klotz et al. did not observe significant daily fluctuations in the clearance of midazolam given as continuous infusion to four healthy volunteers [48]. On the other hand, Koopmans et al. observed circadian changes in midazolam administrated orally to six healthy subjects [49]. The elimination half-life was shortest at 14:00 and longest at 02:00, whereas the sensitivity of the CNS to the drug measured as an α activity of the EEG was greater at 02:00 and 08:00 than at 14:00 and 20:00. However, the differences in the PD were either not significant or on the verge of statistical significance. Further studies concerning chronopharmacology of this drug are required. Orally administrated midazolam is widely used in premedication, whereas when given via intravenous infusion it is used for sedation in ICU settings. Midazolam is also a highly protein-binding (albumin) drug (96-98%), which undergoes significant first-pass oxidative metabolism in the liver and intestine [49,50]. Therefore the potential circadian profile of this drug may differ depending on the route of administration. Circadian changes in the level of plasma albumin may also potentially influence the PK and PD of midazolam.

Opioids

For both weak (tramadol) and strong (morphine and morphinomimetics) opioids clearly substantiated circadian variations in the produced analgesia were demonstrated [51]. In mice, the peak activity of morphine and tramadol was obtained during the activity period [52,53]. As far as human studies are concerned, Auvil- Novack et al. [50] observed the peak and trough demands for morphine or hydromorphone occurred early in the morning and during the night respectively. Also, in other studies the morning peak of opioids delivery using patient controlled analgesia (PCA) was noted [54-56]. In comparison with morning dosing, stronger analgesic effects were reported when tramadol or dihydrocodeine were applied in the evening to relieve healthy volunteers of painful stimuli [57]. There is little information regarding chronopharmacokinetics and chronopharmacodynamics of opioids used during general anaesthesia and during analgosedation in ICU patients, i.e. fentanil, alfentanil, sufentanil and

Table I. Chronopharmacokinetics and chronopharmacodynamics of hypnotics in animals and humans

Authors	Anaesthetics (dose)	Species	Major findings
Fukami et al. (38)	Halothane	Human	The maximum efficacy (minimum consumption) of the agent between 01:00 and 06:00.
Munson et al. (35)	Halothane	Rat	MAC lower at 12:00 (1.26%) than at 20:00 (1.45%).
Scheving et al. (77)	Pentobarbital (35 mg/kg)	Rat	Longer anaesthesia during the rest span (90 *vs.* 35 min).
Rebuelto et al. (31)	Ketamine (40 mg/kg i.p.)	Rat	Longest anaesthesia et 10:00 and 14:00 (rest span) and shortest at 02:00 and 06:00 (activity span).
Naum & Golombek (29)	Etomidate (10-12 mg/kg i.p.) Ketamine with xylazine	Hamster	Significant loss of righting reflex (LORR) at 12:00, no effect at 24:00 for etomidate. No diurnal changes for ketamine anaesthesia.
Rebuelto et al. (32)	Ketamine (40 mg/kg i.p.) + Midzolam (2 mg/kg i.p.)	Rat	Longest response to Ketamine - midazolam during the natural sleep period with the peak at 10:00.
Sato et al. (30)	Ketamine (200 mg/kg, i.p.)	Mouse	The sleep time measured as LORR longest at 22:00; shortest at 10:00 (LD 12h:12h, light on 07:00), no variations in blood concentrations of ketamine.
Sato et al. (34)	Ketamine (200 mg/kg) Pentobarbital (50 mg/kg)	Mouse	The duration of LORR longer at 22:00 than at 10:00; significant differences for ketamine, midazolam and pentobarbital. No variations in the content and activity of Cytochrome P450 enzymes.
Challet et al. (33)	Propofol (100 mg/kg)	Rat	The peak for LORR at 14:10±26 min (the middle of the rest span), no differences in the onset of anaesthesia.
Bienert et al. (39)	Midazolam (50 mg/kg) i.p. for all drug	Human (ICU patients)	No circadian variations in PK and PD (measured using BIS monitor). Severe abnormalities were obtained for BP, HR, body temperature.
Bienert et al. (26)	Propofol (100 mg/kg i.p.)	Rabbit	Circadian variations in PK and PD of propofol, greatest sensitivity to propofol sedation at 16:00, lowest at 10:00 (activity hours), lower propofol clearance at 10:00.
	Propofol (5 mg/kg, 10 min i.v. infusion)		

remifentanil. One of the studies examining these drugs was performed by Gupta et al. who investigated the circadian variations in the clearance of fentanil given to six healthy volunteers via intravenous infusion, finding no diurnal changes [58]. Interestingly, in other studies such fluctuations were observed in pharmacological response to the spinal administration of sufentanil and fentanil [59,60]. Recently, Boom et al. examined the influence of 4 timing moments on fentanil-induced antinociception in healthy volunteers [61]. The peak in pain relief occurred late in the afternoon [17:30] and the trough in the early morning hours [5:30]. No data concerning intravenous sufentanil, alfentanil and remifentanil has been published.

Local anaesthetics

Many studies have demonstrated circadian time-dependent differences in the toxicity, pharmacokinetics and pharmacodynamics of local anaesthetics. The highest toxicity for the amide-type agents was observed in rodents at the beginning of the activity / at the end of the rest phase [51]. The time-dependency in the effectiveness of local anaesthetics was also demonstrated in humans. The longest duration of the effect of lidocaine, mepivacaine and betoxycaine was observed in the afternoon, at 15:00 [62]. Also, the duration of analgesia produced by epidural ropivacaine was greater between 13:00 and 19:00 than at night [63]. Similarly, for spinal

bupivacaine, one peak around noon was observed [64]. Lee et al. tested on 90 patients the chronobiology of 10 mg of intrathecal hyperbaric bupivacaine in three groups 9-12.00, 12-16.00, and 16-8.00. The authors described no differences in peak sensory blockade, duration of motor block down to score I in Bromage scale, nor the adverse effects, but the noon group of patients was characterized by prolonged time of recovery time of sensation to pinprick [65]. Skin permeability to local anaesthetics shows circadian time-dependent difference. Bruguerolle et al. demonstrated temporal variations in the transcutenous passage of lidocaine in children and rats [66]. The plasma concentrations of lidocaine were significantly higher in the evening than at any other time of the day.

Muscle relaxants

The circadian changes in the neuromuscular blocking activity of pancuronium with the lowest response during the activity period have been demonstrated in rats and humans [67]. The concentrations of the drug were not measured in these studies, thus either the PK or cholinesterase activity could be suspected to be influenced by the time-of-day. Recently, Cheeseman et al. [68] noted that time of administration influences the duration of neuromuscular blockade produced by rocuronium. The maximum effect of 50 min. was elicited between 08:00 and 11:00 and the minimum duration of 29 min. was noted between 14:00 and 17:00. This effect is of potential clinical significance and practical relevance. On the other hand, in the very recent study, the Turkish scientists could not detect the circadian periodicity for neuromuscular block induced by vecuronium [69].

However, the circadian profile of other agents, such as cisatracurium, atracurium or mivacurium has not been explored. Similarly, the chronopharmacokinetics of muscle relaxants have not been studied. Potential differences between individual agents may be expected in this field due to differences in elimination (renal, hepatic or by plasma cholinesterases).

Methodological aspects of studying the time-of-day effect of anaesthetics

- **Studies on animals**

Most of studies confirming the circadian rhythms in the area of PK/PD of anaesthetic drugs were conducted on animals. It may be because anaesthetics are very invasive drugs, often with narrow therapeutic indices. It is also much easier to build an animal model and conduct a fully controlled experiments using animal than human. However, we should not forget that an animal model has some limitations and the obtained results may not be simply extrapolated to humans. Using an animal model one should collect as much information as possible about known circadian rhythms of the various physiological functions in order to postulate the possible mechanism of the circadian rhythms in PK/PD of the studied drug.

The rat model is mostly used in chronopharmacological studies. It has a clear nocturnal pattern of activity. Also, circadian fluctuations in various physiological functions are well described on rats. The cardiac output in rats demonstrates diurnal fluctuations with higher values recorded during the dark (activity) phase and lower during the light (rest) phase [70,71]. The diurnal fluctuations in the renal haemodynamics have been studied in rats. During continuous infusion of isotonic saline inuline clearance as well as water and electrolyte excretion were circadian rhythmic, with a nighttime enhancement and daytime minimum [72]. It is also known that the CYP activities fluctuate daily in rats, with high values during the dark period [73,74]. Plasma proteins, such as albumin and α-$_1$ acid glycoprotein as well as plasma endocrine have been documented to be circadian time-dependent [75-77]. Valli et al. [76] studied circadian variations in plasma proteins in an adult male rat. Total proteins, albumin, alpha 2 - and gamma-globulins showed a statistically significant rhythm with a maximum at 04.00 h. Similarly, Scheving et al. [77] found in rats that a crest in total plasma protein occurred between 01:00 and 08:00, with the maximum of 7.1 g/100 ml of plasma at 01:00. Clinically significant consequences of temporal changes in drug binding are relevant for highly bound drugs, especially with a low volume of distribution and a high hepatic extraction ratio. Almost all anaesthetic agents have been shown to act on $GABA_A$. Daily variations in $GABA_A$ receptor function have been described on the cerebral cortex of a hamster with the maximal receptor-binding affinity and $GABA_A$ receptor activity during the activity period [78]. Also, a circadian pattern of the expression of NMDA receptor channels in the brain as well as circadian variation in the number and activity of benzodiazepine receptors has been reported in rats [79,80]. When the rat model is planned for

chronopharmacological studies, the results obtained by Dauchy et al. [75] should be taken into account. They provided compelling evidence that exposure of rats to dim light during the dark phase, as sometimes occurs in laboratory animal facilities, suppresses melatonin production and results in chronobiologic disruptions that can potentially influence the outcome of scientific investigations. The well-known circadian pattern in rodents should enable us to build a good animal model to study the chronopharmacokinetics and chronopharmacodynamics of anaesthetics. However, for full interpretation of the results both the PK and PD of a single drug should be examined.

The rabbit can be a very useful animal to evaluate the PK of different drugs because of its large size, which enables numerous blood samplings. However, rabbits are not very often used in chronobiology, because of an unimodal or bimodal pattern of activity. Their bimodal pattern may be connected with the bimodal expression of the cryptochrome gene Cry 1 observed as early as in neonatal rabbits [81]. Jilge & Hudson claim that the chronobiology of the rabbit seems to be underestimated [82]. One reason for this may be the scattered nature of the available information, making it easy to overlook this species and difficult to form a general view of its circadian biology. Recently a rabbit model has been used to demonstrate the circadian rhythms in the PK and PD of propofol [26]. Also, Choi and Jung observed circadian changes in the PK of sulfamethoxazole orally administrated to rabbits [83]. Another chronobiological study on rabbits by Sato et al. noted nocturnal, comparable to rats, patterns in the circadian rhythms of blood pressure and heart rate [84]. The authors concluded that rabbits could be used in chronobiological studies as well as rats. Rabbits are very sensitive to external influences and under laboratory conditions they may demonstrate a diurnal pattern. Powerful zeitgebers in the rabbit are the light-dark cycle and availability of food. When given scheduled access to food for 4 h/24 h under Light-Dark (LD) 12h:12h, rabbits show activity around the time of food availability. This is the case even when food access is moved from the night to light span, temporarily turning the rabbit into a diurnal animal [82]. Thus, by maintaining the food availability for four morning hours and keeping the animals in LD 12h:12h cycle one may obtain the synchronization of rabbit's rhythms and administration of hypnotics during these hours may be determined by exposure in the activity phase. The diurnal pattern of activity observed in laboratory rabbits may be justified by external influences on the circadian clock [1-3,81]. The retinal clock gates photic inputs and modulates the suprachiasmatic nuclei (SCN) clock [2]. Light simulates Per genes in the SCN and shifts the phase of the circadian rhythm in mammals [85]. Indirect evidence also suggests the existence of a food-entrainable clock outside the SCN [2,86]. The data concerning the circadian rhythmicity of various cardiovascular functions, like cardiac output, volume stroke and/or vascular resistance are limited for rabbits. Sato et al. showed nocturnal pattern in rabbit's blood pressure [BP] and heart rate (HR), however, they did not examine the cardiac output, which may be found as a limitation because there is no direct relationship between BP and CO [84]. On the other hand it may be expected that BP, HR and CO will demonstrate a similar circadian pattern in rabbits, similarly to humans and rats [66,83]. Circadian fluctuations in the liver, lung and intestine metabolizing enzymes have been described in rabbits [88,89]. Rabbit hepatic enzyme activities showed the least obvious rhythmic variations in activity for all three rabbit tissues. The enzyme activities in the intestine showed the peak about 06:00 h and the trough around 12:00-15:00. The second maximum was obtained around the beginning of the dark period. Plasma melatonin was shown to increase during the dark period in rabbits [90]. The same study demonstrated that anaesthesia with a combination of propofol and halothane as well as ketamine anaesthesia both attenuated the release of melatonin whereas pentobarbital had no apparent effect on melatonin. Generally, because of the specific pattern of activity, the chronopharmacokinetics and chronopharmacodynamics of different drugs in rabbits should be examined together with the circadian rhythms of various physiological functions, like BP, HR, body temperature and cortisol or melatonin levels. To determine the activity of animals, daily variations in water intake may be measured [34]. Further studies concerning circadian variations in rabbits' cardiovascular functions, like cardiac output and liver blood flow as well as plasma protein, are required to facilitate interpretation of the results of chronopharmacological studies.

- **Studies on humans**

Studies on the chronopharmacokinetics and chronopharmacodynamics of anaesthetics in humans are limited, especially as far as general anaesthetics

are concerned. Thus, further studies in this field are required. However, some important factors should be taken into consideration when these studies are planned, conducted or interpreted. Some drugs used in anaesthesia are administered via different routes, e.g. opioids, which may be given intravenously or intrathecally, or midazolam given intravenously or orally. Thus, circadian variations in the pharmacokinetics of such a drug may vary depending on the route of administration, because different physiological functions determine the speed and efficacy of individual pharmacokinetic processes. For example, in the case of intravenous administration the processes of absorption and first-pass metabolism are omitted, whereas gastric acid secretion and pH, motility, gastric emptying time and gastrointestinal blood flow vary according to time of the day. Such changes may contribute to time-dependent drug absorption [25,91]. Other pharmacokinetic processes, like distribution, metabolism and elimination are also influenced by the time-of-day. For example, blood flow depends on several regulatory factors including sympathetic and parasympathetic systems, whose activities are known to be circadian time-dependent, with a predominant diurnal effect of the sympathetic system. Thus, the diurnal increase and nocturnal decrease in blood flow may cause differences in drug distribution and metabolism [91-93]. For highly extracted drugs, like propofol or opioids (fentanil, sufentanil), which have flow-dependent clearance, possible circadian rhythms in PK and PD should be connected with daily variations in hepatic blood flow and cardiac output [94,95]. It has been shown that the rate of propofol clearance increased with higher hepatic blood flow, resulting in lower propofol concentrations in the blood [94]. Cugini et al. examined circadian rhythmicity in various cardiovascular functions in healthy subjects and found that the CO had consistent variations during the day, with the minimum of 6 L/min and maximum of 9.49 L/min observed at 17:40 [87]. Similarly to the blood pressure (BP), both the CO and stroke volume (SV) showed day-night differences, with nocturnal minimums. Lemmer & Nold studied the circadian profile of hepatic blood flow, investigated by indocyanine green (ICG) clearance in healthy volunteers and showed the highest values early in the morning and the minimum at 14.00 [96]. Recently Tomalik-Scharte et al. have confirmed that CYP activity may be time-dependent in humans [27]. In conclusion, the eventual circadian fluctuations in the PK or PD of anaesthetic agents obtained for some routes of administration may not be simply extrapolated to other routes. A good example of this is the study conducted by Scott et al., who investigated circadian fluctuations in plasma theophylline given to children (aged 6-17 years) in continuous intravenous infusion and in various sustained-release oral formulations [97]. For the latter the timing of meals must be considered and controlled because feeding conditions contribute to a dosing time-dependent difference in drug absorption [98]. Also, the circadian profile of anaesthetics may differ depending on the population being examined. Different results of chronopharmacological studies of a single anaesthetic agent may be expected if it is given to ICU patients or patients undergoing a surgery. ICU patients demonstrated severe abnormalities in the circadian profile of various physiological functions, such as blood pressure or body temperature and circulating hormones [39,99], which might alter or abolish the possible circadian profile of PK and PD of different drugs. Also, the administration of sedatives per se is thought to suppress temperature rhythms directly by an effect on oscillators, or indirectly through an effect on consciousness [100]. Additionally, some alterations in the concentration of circulating melatonin, cortisol, norepinephrine and aldosterone were observed due to various states of disease and sedative agents given [6,100-102]. An important methodological consideration in the ICU setting may also be constant low level lighting, which may result in a lack of synchronization of an individual patient's circadian clock during the day. Thus, in each of the patients the markers of circadian rhythms, such as core body temperature, plasma melatonin and/or cortisol levels should be continuously measured during the study to enable researchers to analyze and interpret their results. Other factors which may modify the pharmacokinetics and pharmacodynamics of anaesthetics in ICU settings and as a result make difficulties to detect circadian fluctuations may be disease severity of an individual patient. It has been recently demonstrated that propofol clearance in critically ill heart failure patients is decreased by 38%, whereas the sedative effect of propofol is dependent on the severity of illness expressed in terms of the score of sequential organ failure assessment [SOFA] used in the ICU [103]. The one-day surgery may offer the possibility to study the PK and PD of hypnotics on relatively healthy subjects. Thus, in such a study, when an anaesthetic is given to a healthy subject in

a shorter infusion at a different time of the day, possible circadian fluctuations in drug pharmacokinetics and pharmacodynamic response may be more noticeable. Nevertheless, such a study has not been published yet. At least the time-of-day effects of anaesthetics may be different in adults, children and geriatric population. Given that neonates are physiologically and behaviorally arrhythmic for several weeks or months after birth, the results of chronopharmacological studies in this age group are expected to be different than in an adult population. Development of circadian rhythms appears to occur over the first year of life. Plasma melatonin and cortisol levels as well as heart rate have no discernable rhythm within the first days of life, but such daily variations were detected at approximately 3 months of age [104-107]. As far as body temperature is concerned, infants show a nadir similar to adults [03:00h] between 2 and 4 month of age [108-110]. In term neonates circadian cycles may be detected immediately after birth but they subsequently disappear and are not detectable in 3-4 weeks of postnatal life. Therefore, it was suggested that circadian cycles in the early neonatal period are due to maternal influence in utero and that endogenous rhythmicity appears only later [111,112]. Taking into account the fact that the chronopharmacodynamics of anaesthetics may be caused by the similarity between natural sleep and anaesthesia, it is a fact of great importance that during the first months of age some changes in the REM/non-REM sleep cycle were noted. Sleep cycles become more organized and more similar to adults between the age of 3 and 6 months [113]. Also, aging process is connected with some changes in the circadian rhythmicity. A diminished melatonin secretion and a reduced circadian modulation of REM-sleep were observed in older volunteers [114].

PK/PD analysis for assessing the circadian rhythms after single dose and continuous infusion

The fate of a drug and its effects on an organism may vary according to the time of its administration. Very often the time-of-day is a neglected factor in the PK/PD studies, mainly because of experimental efforts needed to fully understand the circadian variation in its PK and PD.

The most popular approach used to assess circadian variations utilizes a set of single dose experiments conducted at different times of a day. This type of experiment allows to determine the relevant PK/PD parameters for each studied period and to statistically evaluate their time-of-day differences. If the difference is significant one can anticipate time-variation in PK/PD parameters of studied drug. This type of approach does not allow to determine the full (24 hr) circadian pattern. The second approach utilizes the steady-state conditions obtained after the constant infusions of a drug. Any 24 hr cycle in a steady-state drug concentrations and/or response is a direct sign of circadian variation.

The mathematical modeling is required to fully understand the circadian rhythms in PK/PD of drugs. It comprises the use of a structural model describing the drug behavior with the inclusion of certain time-dependent parameters that explain the observed circadian variations in the data.

ICU patients are a typical target group treated with prolonged infusion of analgesics and sedatives. On the other hand, in clinical settings many factors may potentially influence the PK and PD of anaesthetic agents, which in consequence may mask or abolish circadian fluctuations. In such conditions population pharmacokinetic/pharmacodynamic modeling with different covariates incorporated and tested may be useful to establish the influence of time of the day on the PK and PD of different drugs. We should not forget that chronobiology includes the influence of external daily rhythms in the environment (e.g., nursing shifts) as well as human biological rhythms. Combining several novel analyses was recently proposed to distinguish the influence of an external rhythm which may incorrectly suggest periodic signals connected with the biological rhythms [115].

Summary

There are a limited number of chronopharmacodynamic studies investigating time-of-day changes in the action of anaesthetic drugs in adults. Carefully conducted studies of this nature have shown clinically significant differences in the duration of action of local anaesthetic agents and muscle relaxants depending on the time-of-day of administration. These findings have not yet led to the tailoring of dosage regimens to compensate for (or take advantage of) these time-of-day effects to improve effectiveness, duration of action, or toxicity profiles. Time-of day has the potential to significantly affect the results of any PK or PD study

and should be included as a covariate of interest. However, the introduction of chronobiology in the field of anaesthesia is sparse and the results are often conflicting. Anaesthetics are being given to patients originating from differential populations, and often suffer from various diseases, which may alter the physiological circadian pattern. Therefore even if the significant time-of-day effect will be confirmed in some patients group, the obtained results may not be simply extrapolated to the other one. Separate studies are needed for different populations.

Conflict of interest
None

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