



## Effect of Day and Night Desflurane Anaesthesia on Melatonin Levels in Rats

Figen Datlı Özer<sup>1</sup>, Elvan Özçmen<sup>1</sup>, Pınar Akan<sup>2</sup>, Hale Aksu Erdost<sup>1</sup>, Sezen Korkut<sup>1</sup>, Ali Necati Gökmen<sup>1</sup>

<sup>1</sup>Department of Anaesthesiology and Reanimation, Dokuz Eylül University School of Medicine, İzmir, Turkey

<sup>2</sup>Department of Biochemistry, Dokuz Eylül University School of Medicine, İzmir, Turkey

**Objective:** The aim of this study is to investigate the effect of day and night administration of desflurane anaesthesia on melatonin levels in rats.

**Methods:** Twenty-four 15-day-old rats were included in the study and were divided into four groups. The rats were anaesthetised between 19:00–01:00 (night group) and 07:00–13:00 (day group) with 5.7% desflurane concentration in 6 L min<sup>-1</sup> 100% oxygen. 6 L min<sup>-1</sup> oxygen was administered to the control groups. At the end of 6 h of anaesthesia, blood samples were taken, and rats were sacrificed. Blood samples were centrifuged and melatonin levels from plasma samples were measured with radioimmunoassay.

**Results:** There was a statistically significant difference between the groups (p=0.007). Between group day control and group night control there was a statistically significant difference (p=0.042). Further, there was a significant difference between group day control and night desfluran as well (p=0.024). We could not find any difference between other groups.

**Conclusion:** This study showed that 6 hours of 5.7% desflurane anaesthesia during day and night hours did not significantly change melatonin levels.

**Keywords:** Melatonin, desflurane, rats

### Introduction

For various reasons, general anaesthesia is frequently used in surgeries for premature babies and very small children. In experimental studies conducted in young/baby animal models, some drugs used in sedation and anaesthesia have been reported to cause histopathological changes in the central nervous system (CNS) and also affect the learning memory functions negatively (1-3). Furthermore, Istaphanous et al. (4) and Kodama et al. (5) demonstrated that volatile anaesthetics have neurotoxic effects on neonatal rats.

Melatonin is a hormone that has anti-inflammatory, oncostatic, antioxidant and anticonvulsant effects with important physiological functions such as the circadian rhythm and reproductive axis regulation (6). The synthesis and secretion of melatonin is stimulated in the dark and is suppressed by light (7). It has been demonstrated that the exogenous application of melatonin is protective against apoptotic neurodegeneration induced by anaesthesia in a developing rat brain, particularly in the cerebral cortex and the anterior thalamus, and has also been reported to increase the levels of Bcl-x protein, which functions as an apoptosis regulator (8).

Anaesthesia and surgical practices affect melatonin secretion and endocrine functions. Furthermore, melatonin production is affected by sleep, pain, medications and stress (9). In the clinical studies conducted so far, general anaesthesia and surgical procedures have been performed during daytime and have had different effects on blood melatonin concentrations (9-11).

In their study about the effects of the anaesthesia procedure performed during daytime compared to that performed during night-time, Özkaya et al. (12) reported that isoflurane anaesthesia increased melatonin levels during daytime whereas it showed no effects during night-time in 15-day-old rats. Dispersyn et al. (13), on the other hand, reported that propofol anaesthesia administered to rats during daytime caused melatonin to create changes in circadian rhythms.

There are no studies that show the effects of night-time desflurane anaesthesia administration on melatonin levels. Therefore, in our study, we aimed to investigate the effects of daytime and night-time desflurane anaesthesia administration on melatonin levels on 15-day-old rats.

## Methods

The study was conducted in Multidisciplinary Laboratory of Experimental Animals after receiving approval from Dokuz Eylül University Animal Experiments Local Ethics Committee (protocol No. 08/2013). The study included 26 Wistar rats in postnatal day 15 (P15), weighing between 15 and 20 g. Since their birth, the rats were observed under a 12-h light (07:00–19:00), 12-h dark (19:00–07:00) cycle.

### Study groups

The rats were randomly divided into four groups;

- Night-time control group (Group NT-C) (n=6): Rats were administered a 6 L min<sup>-1</sup> flow rate of 100% oxygen between 19:00 and 01:00.
- Night-time desflurane group (Group NT-D) (n=7): Rats were administered a 6 L min<sup>-1</sup> flow rate at a 5.7% concentration of desflurane in 100% oxygen between 19:00 and 01:00.
- Daytime control group (Group DT-C) (n=6): Rats were administered a 6 L min<sup>-1</sup> flow rate of 100% oxygen between 07:00 and 13:00.
- Daytime desflurane group (Group DT-D) (n=7): Rats were administered a 6 L min<sup>-1</sup> flow rate at a 5.7% concentration of desflurane in 100% oxygen between 07:00 and 13:00.

**Anaesthesia apparatus and outset:** For each experimental animal, 450 mL glass jars with gas input and output systems were used. A 6 L min<sup>-1</sup> flow rate at a 5.7% concentration of desflurane (Desflurane, Abbott Lab. Istanbul, Turkey) in 100% oxygen input was applied to the glass jars via a vapouriser (14). Monitoring of the gas mixture was stabilised using an anaesthetic gas monitor connected to the common input line (Anaesthesia Gas Monitoring 1304, Denmark). All the jars were placed in a water bath at 37°C, which was maintained constant. It was ensured that the experimental animals breathed the gas mixture for 6 h from these jars. The rats in the night-time groups were kept in the dark during the test to avoid exposure to light. Because rats cannot see in red light, red light was used during the blood-letting procedures.

At the end of the 6-h period when desflurane was discontinued, each rat's chest was quickly opened, and through the left ventricle, 1–3 mL of blood was added to *Eppendorf* tubes, which was then transported to the laboratory via a cold chain.

In the control group, the rats were sacrificed via a cervical dislocation method at the end of the 6-h period, and similar to the other group, each rat's chest was opened, and through the left ventricle, 1–3 mL of blood was added to *Eppendorf* tubes

to determine the melatonin levels. These tubes were then delivered to the laboratory via a cold chain.

**Determining the melatonin levels:** The blood that had been transported to the laboratory was centrifuged for 15 min at +4°C (1000 g) in a cold centrifuge (Hettich Centrifuge Micro R 22, Tuttlingen, Germany). The resulting plasma was taken into Eppendorf tubes and stored at –80°C. Melatonin levels were measured at Dokuz Eylül University Faculty of Medicine, Department of Biochemistry Laboratory, using a rat melatonin radioimmunoassay (RIA) kit (Melatonin Research RIA, Labour Diagnostica Nord GMBH, England).

### Statistical analysis

Statistical analysis was performed using IBM Statistical Package for the Social Sciences version 22.0 (IBM SPSS, Statistics, Armonk, New York, USA). Results are given as the median (min–max) format. In the statistical analysis of the melatonin values, the groups were compared using the Kruskal–Wallis test. For between-group comparisons, the Bonferroni-corrected Mann–Whitney U test was used.  $P < 0.05$  was considered statistically significant.

## Results

All the rats in the study completed the experimental protocol.

The melatonin levels of the groups are given as the median (min–max) in Table 1.

When all the groups were compared using the Kruskal–Wallis test, a significant difference was determined ( $p = 0.007$ ).

When the DT-C and NT-C groups were compared, a significant difference was found ( $p = 0.042$ ). Furthermore, a significant difference was also detected between the DT-C and NT-D groups ( $p = 0.024$ ).

No significant difference was found between the other groups (DT-C x DT-D,  $p = 0.066$ ; NT-C x DT-D,  $p = 0.629$ ; NT-C x NT-D,  $p = 0.652$ ; DT-D x NT-D,  $p = 0.684$ ).

## Discussion

In our study, we found no significant difference in plasma melatonin levels when a 5.7% concentration of desflurane was administered for 6 h to 15-day-old rats during daytime or night-time.

Table 1. Melatonin levels of the groups (pg mL<sup>-1</sup>).

Group	Melatonin median (min–max)
Daytime control	891 (617–1193)
Night-time control	2127 (1193–3000)*
Daytime desflurane	1314 (1011–1581)
Night-time desflurane	2001 (1227–3000)*

\* When groups are compared with the daytime control group,  $p < 0.05$ .  
min: minimum; max: maximum.

Melatonin production is affected by sleep disorders, pain, drugs, stress, surgery and anaesthesia procedures (9). While Castro et al. (15) reported that melatonin secretion increased during propofol infusion, Reber et al. (16) reported that after a 60–90-min isoflurane application, increased melatonin levels continued until the eighth hour. Arai et al. (17) reported that among women the use of isoflurane in anaesthesia induction caused an increase in melatonin levels, but the use of sevoflurane caused a decline. Researchers reported that these results might be related to isoflurane's effect on changing the GABAergic message – which is different from sevoflurane – and decreasing melatonin clearance by reducing the hepatic blood flow, increasing the blood pressure and heart rate and by increasing melatonin synthesis and secretion by sympathetic nervous system activation. Yağar et al. (18) failed to detect differences in melatonin levels of patients who had undergone cardiac surgeries at different times of the day. However, the time intervals in their study were selected as 08:00–09:00, 11:00–13:00 and 16:00–19:00.

Melatonin secretion occurs by means of stimulating norepinephrine  $\alpha$  and  $\beta$  receptors via sympathetic stimulation (19). Darkness stimulates the release of norepinephrine, which activates  $\beta$ 1-adrenergic receptors in the pituitary gland, through retinal photoreceptors, increases cyclic AMP levels and stimulates melatonin synthesis, which is catalysed by the enzyme. Desflurane, on the other hand, causes an increase in heart rate and tachycardia due to sympathetic system activation (20). Melatonin increase might be expected due to the stimulation of  $\beta$ 1-adrenergic receptors in the pituitary under the influence of desflurane's sympathetic activation; however, we did not detect any evidence of this in our study.

The circadian rhythm is disrupted during general anaesthesia. It has been shown in different studies that melatonin levels are differently affected by anaesthesia and surgery (9–11). The different melatonin levels detected in studies might be explained by anaesthesia application, the additional use of other drugs or differences between the measurement times of melatonin levels and surgery. According to the results obtained in all these studies, the effect of anaesthesia on melatonin secretion could not be determined precisely, and the effect of surgery could not be ruled out. In this study, it was important to check only the impact of anaesthesia on melatonin levels without surgery or the application of any painful stimuli to understand the change that desflurane alone can cause on melatonin levels.

Melatonin secretion is dependent on light intensity (21) and has a pronounced circadian rhythm (22). Reber et al. (16) reported that creating a dark setting for an anaesthesia application performed during daytime increased melatonin levels. In contrast, in our study, no increase in melatonin levels was observed after desflurane administration. Unlike the results we obtained from our study, Özkaya et al. (12) reported that isoflurane application during daytime increased the plasma

concentration of melatonin. Their explanation for these results was that the dark setting formed by daytime anaesthesia application increased melatonin secretion and thus caused an increase in plasma levels. We believe that these different results were caused by GABA and NMDA receptor's being exposed to various doses of inhalation agents that were utilised and, therefore, their different effects secretion occurred.

Desflurane has been reported to have an impact by increasing GABA-inhibitory function via GABA receptors (23). In addition, GABA receptors were detected in many areas of the brain linked with the suprachiasmatic nucleus (SCN) (24). It has been demonstrated that GABAergic agonists, such as *muscimol*, triazolam and phenobarbital, induced a phase shift of the circadian rhythm (25, 26) by modifying the expression of CLOCK genes, such as *per1* and *per2*, in SCN. Cheeseman et al. (27) reported that isoflurane anaesthesia applied on honey bees during daytime caused a deviation in time perception by affecting the *per*, *cry* and CLOCK genes.

The expression of CLOCK genes can be suppressed through the activation of GABA receptors in the SCN, and this suppression may be a mechanism for the phase shift formation in the circadian rhythm formed by anaesthetics. Moreover, Dispersyn et al. (13) monitored the decrease in melatonin secretion in the first 3 h and the increase at 20<sup>th</sup> h after anaesthesia termination following a 30-min propofol anaesthesia administration in rats and stated that this could be related to a shift in the circadian rhythm of melatonin. However, we could not detect this effect of anaesthesia in our study.

The reason for the night-time desflurane application causing a lack of significant melatonin increase might be related to melatonin's demolition being fast due to the function of the enzymes in melatonin metabolism being increased in the darkness period (28) and/or due to night-time anaesthesia application causing a lower phase shift.

The limitation of this study might be that the plasma melatonin levels cannot be viewed for 24 h or longer. Further, the baby rats included in the study weighed between 15 and 20 g and did not have a sufficient amount of blood for repeated measurements of melatonin levels (54–70 mL kg<sup>-1</sup>) (29). Therefore, we had to evaluate the changes in plasma melatonin levels in a single measurement. Furthermore, melatonin is not stored by the pineal gland, and melatonin enters the circulation immediately after being synthesised (7). However, it is not precisely known at which hour anaesthesia-induced melatonin secretion takes place during anaesthesia or how soon it takes to detect it in the blood. Moreover, one of the other limitations of this study is that we did not perform repeated measurements to see whether this effect is more increased after anaesthesia is terminated or to show how long it continues. Although Özkaya et al. (12) demonstrated that melatonin level increased following daytime anaesthesia application in samples that had been immediately taken after isoflurane anaesthesia; we could not confirm such an effect of

desflurane in this study. Desflurane may not affect melatonin levels, but it may cause a later increase. We might have been unable to detect this effect as we took only one sample at the end of anaesthesia.

The inability to measure the sympathetic response formed by desflurane is also one of the limitations of our study. New studies are required to show whether it is sleep caused by desflurane anaesthesia applied during the daytime or whether desflurane causes a phase shift in the circadian rhythm of melatonin for other reasons (such as GABA receptors and sympathetic activation.) and to show the relationship with CLOCK genes, such as *per*, *bmal* or *clock*. In this study, we showed that daytime and night-time applications of desflurane anaesthesia in 15-day-old rats with a light–dark (12 h/12 h) cycle have no effect on the secretion of melatonin. From a clinical point of view, our results suggest that melatonin is not responsible for changes such as sleep disorders and postoperative cognitive dysfunctions (30) resulting from an increase in plasma melatonin levels due to anaesthesia administration independent of surgeries and in which the circadian rhythm is not disrupted because of desflurane anaesthesia.

## Conclusion

In this study performed on 15-day-old rats, we found that 5.7% desflurane concentration administered over a 6-h daytime or night-time period did not have an effect on melatonin levels. However, further studies are required before chronopharmacologic activities related to general anaesthetic agents are put into anaesthesia practice.

**Ethics Committee Approval:** Ethics committee approval was received for this study from the ethics committee of Dokuz Eylül University.

**Peer-review:** Externally peer-reviewed.

**Author Contributions:** Concept - A.N.G.; Design - A.N.G., E.Ö.; Supervision - A.N.G.; Resources - A.N.G.; Materials - A.N.G., P.A., F.D.Ö.; Data Collection and/or Processing - F.D.Ö., E.Ö., H.A.E., S.K.; Analysis and/or Interpretation - F.D.Ö., E.Ö., H.A.E., P.A., A.N.G.; Literature Search - F.D.Ö., E.Ö.; Writing Manuscript - F.D.Ö., E.Ö.; Critical Review - E.Ö., H.A.E., A.N.G.; Other - F.D.Ö., E.Ö., H.A.E., A.N.G.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study has received no financial support.

## References

- Jevtovic-Todorovic V, Hartman RE, Izumi Y, Benshoff ND. Early exposure to common anesthetic agents causes widespread neurodegeneration in the developing rat brain and persistent learning deficits. *J Neurosci* 2003; 23: 876-82. [\[CrossRef\]](#)
- Ikonomidou C, Bittgau P, Koch C, Genz K, Hoerster F, Felderhoff-Mueser U, et al. Neurotransmitters and apoptosis in the developing brain. *Biochem Pharmacol* 2001; 62: 401-5. [\[CrossRef\]](#)
- Loepke AW, Soriano SG. An assessment of the effects of general anesthetics on developing brain structure and neurocognitive function. *Anesth Analg* 2008; 106: 1681-707. [\[CrossRef\]](#)
- Istaphanous GK, Howard J, Nan X, Hughes EA, McCann JC, McAuliffe JJ, et al. Comparison of the neuroapoptotic properties of equipotent anesthetic concentrations of desflurane, isoflurane, or sevoflurane in neonatal mice. *Anesthesiology* 2011; 114: 578-87. [\[CrossRef\]](#)
- Kodama M, Satoh Y, Otsubo Y, Araki Y, Yonamine R, Masui K, et al. Neonatal desflurane exposure induces more robust neuroapoptosis than do isoflurane and sevoflurane and impairs working memory. *Anesthesiology* 2011; 115: 979-91. [\[CrossRef\]](#)
- Naguib M, Gottumukkala V, Goldstein PA. Melatonin and anesthesia: a clinical perspective. *J Pineal Res* 2007; 42: 12-21. [\[CrossRef\]](#)
- Reiter RJ. The Pineal Gland. *Principles of Medical Biology* 1998; 10: 145-64. [\[CrossRef\]](#)
- Yon JH, Carter LB, Reiter RJ, Jevtovic-Todorovic V. Melatonin reduces the severity of anesthesia-induced apoptotic neurodegeneration in the developing rat brain. *Neurobiol Dis* 2006; 21: 522-30. [\[CrossRef\]](#)
- Ram E, Vishne T, Weinstein T, Beilin B. General anesthesia for surgery influences melatonin and cortisol levels. *World J Surg* 2005; 29: 826-9. [\[CrossRef\]](#)
- Cronin AJ, Keifer JC, Davies MF, King TS. Melatonin secretion after surgery. *Lancet* 2000; 356: 1244-5. [\[CrossRef\]](#)
- Karkela J, Vakkuri O, Kaukinen S, Huang WQ. The influence of anaesthesia and surgery on the circadian rhythm of melatonin. *Acta Anaesthesiol Scand* 2002; 46: 30-6. [\[CrossRef\]](#)
- Özkaya F, Öçmen E, Akan P, Calan Ö, Özel Ö, Ergürz S, ve ark. Ratlarda Gece veya gündüz izofluran uygulaması Plazma Melatonin Düzeyini Etkiler. *Türk J Anaesthesiol Reanim* 2013; 41: 111-4.
- Dispersyn G, Pain L, Touitou Y. Propofol anesthesia significantly alters plasma blood levels of melatonin in rats. *Anesthesiology* 2010; 112: 333-7. [\[CrossRef\]](#)
- Steffey EP. Inhalation anesthetics, In: Lumb and Jones' veterinary anesthesia 4rd ed, (Thurman J, Tranquilli W, Benson J, eds). Baltimore, Williams & Wilkins. 355-93.
- Castro MR, Pastor AB, Alcantud JF, Salvan JH. Altered plasma melatonin concentrations after administration of propofol in continuous infusion. *Rev Esp Anesthesiol Reanim* 2007; 54: 469-74.
- Reber A, Huber PR, Ummenhofer W, Gürtler CM. General anaesthesia for surgery can influence circulating melatonin during daylight hours. *Acta Anaesthesiol Scand* 1998; 42: 1050-6. [\[CrossRef\]](#)
- Arai YC, Ueda W, Okatani Y, Fukaya T. Isoflurane increases, but sevoflurane decreases blood concentrations of melatonin in women. *J Anesth* 2004; 18: 228-31. [\[CrossRef\]](#)
- Yağar S, Altınkaya Çavuş M, Dönmez A, Karadeniz Ü, Açık G, Saydam GS, ve ark. Açık Kalp Cerrahisi Geçiren Hastalarda Anestezi Zamanının Plazma Kortizol ve Melatonin Düzeylerine Etkisi Var mı? *GKDA Derg* 2015; 21: 77-81.
- Buscemi N, Vandermeer B, Hooton N, Pandya R, Tjosvold L, Hartling L, et al. Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction: meta-analysis. *Br Med J* 2006; 332: 385-93. [\[CrossRef\]](#)
- Arain SR, Shankar H, Ebert T. Desflurane enhances reactivity during the use of the laryngeal mask airway. *Anesthesiology* 2005; 103: 495-9. [\[CrossRef\]](#)

21. McIntyre IM, Norman TR, Burrows GD, Armstrong SM. Human melatonin suppression by light is intensity dependent. *J Pineal Res* 1989; 6: 149-56. [\[CrossRef\]](#)
22. Bojkowski CJ, Arendt J, Shih MC, Markey SP. Melatonin secretion in humans assessed by measuring its metabolite, 6-sulfatoxymelatonin. *Clin Chem* 1987; 33: 1343-8.
23. Campagna JA, Miller KW, Forman SA. Mechanisms of actions of inhaled anesthetics. *N Engl J Med* 2003; 348: 2110-24. [\[CrossRef\]](#)
24. O'Hara BF, Andretic R, Heller HC, Carter DB. GABAA, GABAC, and NMDA receptor subunit expression in the suprachiasmatic nucleus and other brain regions. *Brain Res Mol Brain Res* 1995; 28: 239-50. [\[CrossRef\]](#)
25. Ehlen JC, Novak CM, Karom MC, Gamble KL. GABAA receptor activation suppresses Period 1 mRNA and Period 2 mRNA in the suprachiasmatic nucleus during the mid-subjective day. *Eur J Neurosci* 2006; 23: 3328-36. [\[CrossRef\]](#)
26. Legan SJ, Donoghue KM, Franklin KM, Duncan MJ. Phenobarbital blockade of the preovulatory luteinizing hormone surge: association with phase-advanced circadian clock and altered suprachiasmatic nucleus Period 1 gene expression. *Am J Physiol Regul Integr Comp Physiol* 2009; 296: 620-30. [\[CrossRef\]](#)
27. Cheeseman JF, Winnebecka EC, Millar CD, Kirkland LS, Sleight J, Goodwind M, et al. General anesthesia alters time perception by phase shifting the circadian clock. *PNAS* 2012; 109: 7061-6. [\[CrossRef\]](#)
28. Ghouri AF, White PF. Effect of fentanyl and nitrous oxide on the desflurane anesthetic requirement. *Anesth Analg* 1991; 72: 377-81. [\[CrossRef\]](#)
29. Soylu SM. Rat Fizyolojisi. In: Küçük Deney Hayvanlarından Rat. Eds Orhan Yücel. Ankara 2012, p.22-5.
30. Wu Y, Wang J, Wu A, Yue Y. Do fluctuations in endogenous melatonin levels predict the occurrence of postoperative cognitive dysfunction (POCD)? *Int J Neurosci* 2014; 124: 787-91. [\[CrossRef\]](#)