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Propofol Anesthesia Significantly Alters Plasma Blood Levels of Melatonin in Rats

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ABSTRACT

Background: General anesthesia combined with surgery has been shown to decrease the nocturnal peak of melatonin in patients. However, the role of anesthesia itself on melatonin secretion remains unknown. We previously showed that anesthesia induced by propofol modifies the circadian time structure in both rats and humans and phase advances the circadian rest-activity rhythm in rats. In this study, we examined the secretion of melatonin during 24 h after a 30-min propofol anesthesia in rats.

Methods: Rats were exposed to 12-h light/12-h dark alteration conditions and anesthetized with propofol (120 mg/kg intraperitoneally) around their peak of melatonin secretion (Zeitgeber time 16). Trunk blood samples were collected at seven subsequent Zeitgeber times to assess the effects of propofol on circadian melatonin secretion.

Results: Propofol modifies the peripheral melatonin by significantly decreasing its concentration (~22–28%) during the immediate 3 h after the wake up from anesthesia and then significantly increasing melatonin secretion 20 h after anesthesia (~38%). Cosinor analysis suggests that propofol induces a phase advance of the circadian secretion of peripheral melatonin.

Conclusions: The results demonstrate the disturbing effects of propofol anesthesia on the circadian rhythm of plasma melatonin in rats under normal light conditions. These results parallel the desynchronization of the circadian rhythms of locomotor activity and temperature previously observed after propofol anesthesia.

What We Already Know about This Topic

- Brief propofol anesthesia disrupts the circadian sleep structure in animals and humans
- Surgery and anesthesia disrupt circadian sleep structure and timing of melatonin release, but the effect of anesthesia alone is not known

What This Article Tells Us That Is New

- Propofol anesthesia resulted in a phase advance of secretion of melatonin, suggesting that the disruption of sleep after surgery reflects in part the effects of anesthetics themselves

MELATONIN is a nocturnal hormone mainly released by the pineal gland and acts as a photoneuroendocrine transducer of information on day length.[1] Pineal melatonin synthesis is under the sympathetic control from the central circadian pacemaker located in the suprachiasmatic nuclei (SCN).[2] The SCN control the circadian rhythm of rest activity, temperature, and melatonin secretion. These circadian rhythms are synchronized with the light–dark cycle; and the peripheral melatonin secretion provides circadian information to the rest of the body.[3] Therefore, peripheral melatonin secretion is considered as a biomarker of circadian rhythmicity and is altered in some particular situations of circadian dysregulation, such as the shift work condition[4–6] and the time zones crossing, the "jet-lag phenomenon."[5,7]

General anesthesia is an altered state of consciousness,[8,9] which may have some impact on the circadian structure.[10] Propofol is one of the most widely used intravenous agents because of its pharmacokinetic advantages. In a series of experiments, we previously showed that propofol anesthesia alone (without surgery) disturbed the circadian structure in rodents as well as in humans.[10–12] We observed a phase advance of the circadian rhythms of body temperature and rest activity after propofol anesthesia in rats under various laboratory conditions,[10,12] whereas the circadian sleep-wake activity was desynchronized to local time, the days after anesthesia in patients in real life conditions.[11] Our hypothesis was that propofol anesthesia could equally disturb the circadian rhythm of melatonin.

In this field, general anesthesia combined with surgery decreases the nocturnal peak of melatonin or decreases the total amount of melatonin metabolites in patients.[3,13–16] Impaired melatonin secretion has been proposed as one of the

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mechanisms involved in the postoperative sleep disorders observed in patients undergoing anesthesia and surgery.[15] The difficulty in all these clinical studies is that general anesthesia (using different anesthesia regimens) is always performed in conjunction with surgery and premedications. Thus, it is impossible to distinguish the effects of general anesthesia from those of surgery and other perioperative treatments.[17]

The aim of our study was to determine whether propofol anesthesia (without surgery and other medications) disturbs the melatonin secretion during the 24-h postanesthesia period. For this purpose, we examine the effects of an anesthetic dose of propofol on the secretion of peripheral melatonin during 24 h in rats under normal light–dark alternation conditions.

Materials and Methods

This study, including care of the animals involved, was conducted according to the official edict presented by the French Ministry of Agriculture (Paris, France) and the recommendations of the Declaration of Helsinki. Thus, these experiments were conducted in an authorized laboratory and under the supervision of an authorized researcher (Y.T.). All research procedures were also performed in agreement with standards and ethics for animal biologic rhythm research.[18]

Animals

Eighty-four male Wistar rats (Janvier, Le Genest-Saint-Isle, France) weighing 200 to 260 g (5-week old) were housed in individual cages at the beginning of the experiments, with food and water available *ad libitum*, and maintained in a chronobiologic animal facility (Enceinte Autonome d'Animalerie, Ref. A 110-SP-6, ESI Flufrance, Arcueil, France). The chronobiology facility was equipped with equidistant, sound-proof, temperature-controlled (21° ± 1.0°C) compartments, each provided with independent light–dark cycles. Rats were maintained for 3 weeks in a 12-h light/12-h dark cycle (L/D 12:12) before the start of the experiment.

Drugs

The anesthetic regimen of propofol was conforming to previous published data obtained for rats.[19,20] Propofol (10 mg/ml; 120 mg/kg; Fresenius Kabi, Sèvres, France) or control solution (intralipids 10%, 10 ml/kg; Fresenius Kabi) was administered intraperitoneally. In Wistar male rats, the duration of general propofol anesthesia (120 mg/kg) ranged between 25 and 30 min. All rats have recovered their righting reflex 30 min after propofol administration.

Experimental Procedures

Rats were divided into 14 groups (n = 6 animals per group) according to the treatment received (propofol or intralipids) and the time of blood samples' collections at +1, +4, +8, +12, +16, +20, and +24 h after propofol or intralipids administration. Thus, for each time point, we injected 12 rats (half with propofol and half with intralipids).

Fig. 1. Drug administration. Rats received intraperitoneal injection of propofol (n = 42) or intralipids (n = 42) at one Zeitgeber time (ZT16). ZT0 represents the beginning of the rest period (light onset) and ZT12 the beginning of the activity period (light offset).

Propofol or intralipids were administered at one Zeitgeber time (ZT) ZT16. In circadian terminology, ZT0 and ZT12 define light on (offset of activity period) and light off (onset of activity period), respectively (fig. 1). Thus, rats were injected 4 h after the onset of the activity period in nocturnal rodents.

Melatonin Measurement

Rats were killed by decapitation. Trunk blood samples were collected in plastic tubes with heparin. Samples were kept on ice, centrifuged at 4°C (4,000 *g*) for 15 min, and the plasma was stored at −40°C until assay.

Plasma melatonin was measured after dichloromethane extraction by radioimmunoassay with a specific rabbit antiserum (R19540; INRA, Nouzilly, France) and ^{125}I-labeled melatonin tracer (Perkin-Elmer, France) according to the method of Brown *et al.*[21] Two hundred microliters of samples were used for the assay. The sensitivity of the melatonin assay was 5 to 10 pg/ml. The intraassay coefficients of variation were 4, 7, and 10%, and the interassay coefficients of variation were 10, 9, and 15% for melatonin concentrations 50, 200, and 1000 pg/ml, respectively.

Statistical Analysis

Statistical analysis of data was performed using two-way analysis of variance (between factor: ZT of blood sample [ZT17, ZT20, ZT24, ZT4, ZT8, ZT12, and ZT16] and treatment [propofol *vs.* intralipids]; dependent factor: melatonin concentration). At each time point, multiple comparisons between propofol and intralipids were performed using *t* test with Bonferroni correction. Significance was defined as $P < 0.05$.

Melatonin is secreted according to a circadian (nearly 24 h) rhythm. In rodents, melatonin decreases to its lowest concentrations at ZT0, which corresponds to the offset of activity period, and reaches its highest concentrations during the early hours of the activity period (between ZT12 and ZT16). This variation may be fit to a sinusoidal function by the cosinor method, a linear method of least squares. Cosinor analysis detects a circadian rhythm when the rhythm amplitude is different from zero with a $P < 0.05$.[22] Propofol not only modifies the peripheral blood melatonin at different time points but also disrupts its circadian rhythm. Any shift in the circadian rhythm (phase advance or phase delay) has a pro-

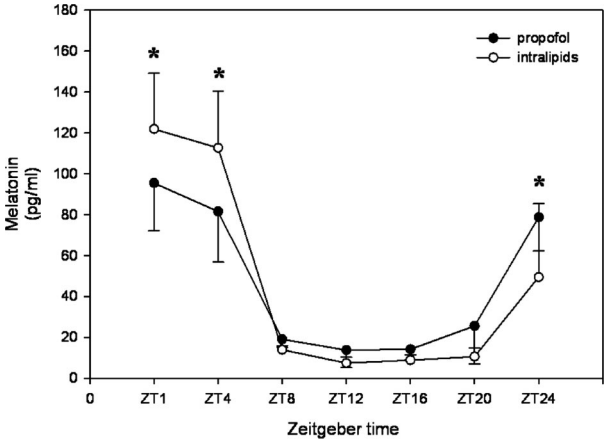

Fig. 2. Effects of propofol anesthesia or control treatment on melatonin secretion at one Zeitgeber time of injection (ZT16) on peripheral melatonin secretion. Values are given as mean ± SD. * Significant differences ($P < 0.05$) to mean values between propofol and control.

found effect on the regulation of sleep–wake cycle and could lead to sleep disorders.[4,5,7] To examine a putative effect of propofol on the circadian rhythm of peripheral melatonin, cosinor analysis and comparison of linear regressions for the two treatments (intralipids and propofol) were performed using SigmaPlot software (Jandel Scientific, Chicago, IL).

Results

A significant modification of melatonin secretion was observed after propofol administration at ZT16 when compared with control administration (fig. 2). Two-way analysis of variance detected a significant effect of time ($F[6,63] = 64.13$; $P < 10^{-4}$) and no effect of treatment ($F[1,63] = 0.018$), but a significant interaction between treatment and time ($F[6,63] = 3.906$; $P < 0.002$). *Post hoc* analysis showed significant differences at ZT17 ($P = 0.015$), ZT20 ($P = 0.004$), and ZT16 ($P = 0.019$). For the first two ZTs after injections (ZT17 and ZT20), propofol significantly decreased melatonin secretion. On the contrary, propofol increased melatonin secretion 24 h after the propofol administration at ZT16. Cosinor analysis detected circadian rhythms of peripheral melatonin for both treatments: propofol ($F[2,39] = 48.62$, $P < 10^{-4}$) and intralipids ($F[2,36] = 30.46$, $P < 10^{-4}$). Comparison between treatments revealed only a trend with a 40-min (CI 95%: 1–81 min; $P = 0.068$) phase advance melatonin peak cosinor parameter (ZT in h:min ± SD in min) after propofol anesthesia when compared with intralipids (ZT 17:50 ± 27 *vs.* ZT 18:30 ± 31 for propofol and intralipids, respectively; fig. 3).

Discussion

This study shows that general propofol anesthesia decreases the plasma melatonin concentration during the first 4 h after anesthesia and increases the melatonin secretion 20 h after anesthesia. These results demonstrate the disturbing effects of general propofol anesthesia on the circadian rhythm of melatonin in rats under usual alternation of light and darkness.

Many clinical studies suggest that general anesthesia impacts melatonin secretion, although this has never been demonstrated. Importantly, most of the studies have been done when general anesthesia was combined with surgery. General anesthesia for hysterectomy surgery was associated with a decrease in melatonin secretion on the first night after anesthesia.[15] The amount of 6-sulfatoxymelatonin, the urinary metabolite of melatonin, decreased significantly on the first postoperative night after thiopental or isoflurane anesthesia for orthopaedic surgery when compared with the preopera-

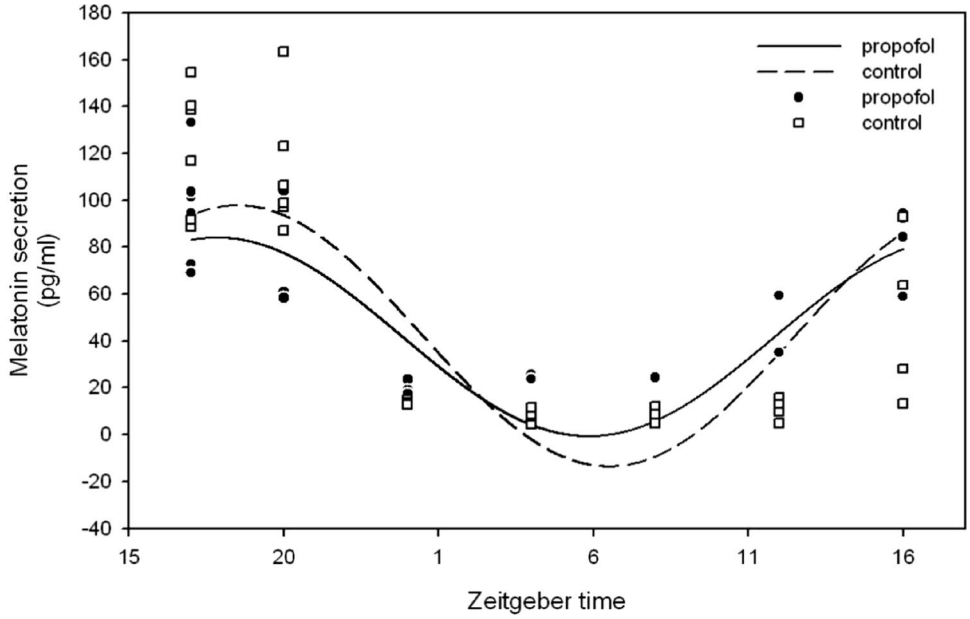

Fig. 3. Cosinor analysis of data of melatonin secretion during 24 h after propofol or intralipids administration at Zeitgeber time 16. Circadian rhythm of peripheral melatonin was observed for both treatments, with a mean shift of 40 min for the peak cosinor parameter (see text for explanation).

tive night.[3] The plasma melatonin concentration has been shown to decrease under general anesthesia and surgery[16] but seemed to be unchanged during the first hours after general anesthesia and surgery.[23] Conversely, two studies showed an opposite effect. General anesthesia (thiopental and fentanyl or propofol and fentanyl) increased melatonin secretion during anesthesia[24] or during the 8 h after general anesthesia for surgery.[14] Finally, the melatonin concentration was unchanged immediately after thiopental or midazolam injection in children.[25] Such differences from one study to another could be easily explained by differences in anesthesia regimen, co-medications, surgeries performed, and time of sampling for melatonin concentration assessment. From all these previous results, the effect of general anesthesia on melatonin secretion itself remains undetermined.

The decrease in melatonin secretion immediately after propofol anesthesia was followed by an increase in melatonin secretion 20 h after the wake up from anesthesia. The results suggest that this increase may be due to a shift in the circadian rhythm of melatonin secretion (figs. 2 and 3). In a series of experiments, we recently demonstrated that general propofol anesthesia *per se* (without surgery and premedication) disturbed the circadian pattern of the rest-activity rhythm during the first postanesthesia days in both rats and humans.[10–12] Propofol given at an anesthetic dose in rats induced a phase advance of the rest-activity and temperature rhythms.[10,12] Melatonin secretion and rest-activity rhythm are controlled by the same circadian pacemaker (SCN).[16,26] Thus, general anesthesia can theoretically create a phase advance in the circadian rhythm of melatonin, which is similar to the phase advance previously observed for the circadian rhythms of rest activity and temperature. In this study, we examined the effects of propofol on plasma melatonin concentration by using seven sampling times during a 24-h period. Our result showed a 40-min phase advance in the circadian melatonin rhythm in the animals submitted to propofol anesthesia when compared (statistical trend) with the control situation (intralipids). From a methodological point of view, we may have missed the onset of melatonin secretion after anesthesia by the limited seven sampling times examined or the statistical significance because of the higher amplitude of the circadian rhythm of melatonin.

Propofol acts *via* a positive modulation of the inhibitory function of the neurotransmitter γ-aminobutyric acid (GABA) through GABA receptors type A ($GABA_A$). $GABA_A$ receptors are expressed in many brain areas, including the outputs from SCN.[27] Indeed, GABA seems to be involved in transmitted signals from the SCN to the paraventricular nucleus stimulation of the melatonin synthesis from pineal gland. Infusion of GABA during the activity period inhibits melatonin secretion, whereas infusion during the rest period decreases melatonin synthesis.[28] However, recent data showed that GABAergic agonists such as muscimol, triazolam, and phenobarbital induce phase advance of circadian rhythm, whereas they modify the expression of genes clock such as *per*1 and *per*2 within the SCN.[29,30] The activation of $GABA_A$ receptors within the SCN may inhibit the clock genes expression and may constitute a common mechanism for these drugs to induce a phase advance of the circadian rhythms. One hypothesis is that propofol, agonist GABA, has an inhibitory effect on the outputs from SCN to paraventricular nucleus. The alternative mechanism might be a direct effect on clock genes expression within the SCN, which induces a phase advance of the circadian rhythms, including the circadian melatonin peripheral secretion. This is currently under examination.

Propofol anesthesia clearly disturbed the endogenous melatonin rhythm in rodents. These results extend and emphasize the effects of propofol on circadian markers. Indeed, the resetting effect of propofol anesthesia on internal clock, as observed in specific laboratory conditions,[10] seems to be responsible not only for a desynchronization of rest-activity rhythms to local time in both rodents[12] and patients[11] but also for a desynchronization of peripheral melatonin secretion. Melatonin is a key marker to provide day-length information to the body and is especially involved in the regulation of the sleep–wake cycle.[31] A desynchronization of the sleep–wake rhythm to the local time is well known to be associated with altered vigilance and obvious health consequences (fatigue). However, the circadian rhythm of melatonin is also closely associated with human mood and performance.[32] In anaesthesiology, and more particularly in ambulatory practice, the quality of recovery has to be considered in terms of health and economic factors. Any disturbance of the peripheral melatonin rhythm is, therefore, clinically important. Unexplained clinical symptoms occur in the days after anesthesia, including fatigue, drowsiness, sleep disorders, and mood alteration even for a short-duration anesthesia for a minor surgical procedure. Such complaints from the patients might be sustained at least by a disturbance in melatonin secretion induced by propofol anesthesia.

There are a number of limitations in this study. Rats are nocturnal animals. Therefore, the peak of melatonin occurs in rats during the activity period, whereas it takes place during the rest period in humans. We acknowledge that our results on rats cannot be directly applied to propofol effects on human melatonin. However, the time chosen for this study mimicked the time period when anesthesia is largely performed in patients, that is, the activity period. This is the reason why we examined the impact of rat propofol anesthesia on the peak of melatonin in the activity period. From our data obtained in rats, we cannot demonstrate that the fatigue, drowsiness, and sleep disorders observed in patients are related to a disturbed circadian pattern of human melatonin. However, our results open new lines of researches to better understand and perhaps correct these symptoms.

However, our study demonstrates for the first time that propofol administered at an anesthetic dose impacts the circadian secretion of melatonin during at least 24 h after anesthesia in rats submitted to light–dark alternation. From a clinical point of view, propofol-induced dysregulation of circadian mel-

atonin rhythm might be one factor that impairs recovery from anesthesia, independent of the surgical procedure.

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