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EFFECTS OF VOLATILE ANESTHETICS ON THE CIRCADIAN RHYTHMS OF RAT HIPPOCAMPAL ACETYLCHOLINE RELEASE AND LOCOMOTOR ACTIVITY

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Abstract—General anesthesia is occasionally associated with postoperative complications such as sleep disorder, drowsiness, or mood alterations. Hippocampal acetylcholine (ACh), the extracellular level of which increases during the dark (active) phase and decreases during the light (rest) phase in rats, is thought to be associated with locomotor activity and be crucial for learning and memory. Propofol, an intravenous anesthetic, is known to shift the circadian rhythms of physiological parameters including locomotor activity and body temperature in both rodents and humans, while the effects of volatile anesthetics on the circadian rhythm largely remain unclear. The present study examined the effects of isoflurane anesthesia on the diurnal changes in hippocampal ACh release and locomotor activity in rats. Rats were divided into three groups: a light-phase anesthesia group (LA group), a dark-phase anesthesia group (DA group), and a control group. They were exposed to a 12-h light/12-h dark environment and anesthetized with 1.4% isoflurane for 4 h during the middle of the light phase (LA group) and dark phase (DA group). Simultaneous measurement of hippocampal ACh by microdialysis and locomotor activity were done for 60 h under free-moving conditions. Hippocampal ACh release and locomotor activity showed a clear circadian rhythm. In the DA group, but not in the LA group, the diurnal variation in ACh release was significantly disturbed and a more than 2-h phase-advance in locomotor activity was observed. There was a significant correlation between hippocampal ACh release and locomotor activity, and isoflurane anesthesia disrupted it even after anesthesia was discontinued. This study revealed that the levels and circadian rhythms of hippocampal ACh release and locomotor activity were more sensitive to isoflurane anesthesia when it was administered during the active phase. Our findings suggest that anesthesia exerts differential effects on the regulation of circadian rhythms depending on the circadian phase.

Key words: circadian rhythm, hippocampal acetylcholine, locomotor activity, isoflurane anesthesia, *in vivo* microdialysis.

INTRODUCTION

A number of body functions such as body temperature, blood pressure, hormone secretion, sleep and activity, and gastric and renal function have circadian rhythms (i.e. temporal variations within a roughly 24-h cycle) (Chassard and Bruguerolle, 2004). Disturbances in these circadian rhythms might be associated with common postoperative complications in the central nervous system, such as sleep disorders, delirium, and cognitive dysfunction (Rasmussen et al., 2005), which could lead to increased morbidity and mortality (Pratico et al., 2005).

Clinical studies have shown that anesthesia with or without major surgery results in circadian rhythm disturbances in hormone secretion and rest–activity rhythm (Karkela et al., 2002; Rasmussen et al., 2005). Propofol alone (without surgery) affects the circadian rhythms in rodents even though its pharmacokinetic properties allow rapid elimination (Challet et al., 2007; Dispersyn et al., 2008; Dispersyn et al., 2009a,b).

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Abbreviations: ACh, Acetylcholine; aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; DA, dark phase anesthesia; LA, light phase anesthesia; GABA, γ-aminobutylic acid.

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Acetylcholine (ACh), one of the major neurotransmitters in the central nervous system, is known to play important roles in arousal state and consciousness (Lydic and Baghdoyan, 2005). Especially, hippocampal ACh has been revealed to be critical for learning, memory, and cognitive function (Day et al., 1991; Levin and Simon, 1998; Ihalainen et al., 2011). Previous studies using microdialysis in rodents showed diurnal changes in hippocampal ACh release (Mizuno et al., 1991) and that spontaneous hippocampal ACh release is higher during the dark (active) phase than during the light (rest) phase in nocturnal mammals (Jimenez-Capdeville and Dykes, 1993; Mitsushima et al., 2009). Additionally, although the causal relationship between hippocampal ACh secretion and locomotion activity has not yet been clarified, previous studies have indicated that there is a correlative relationship between hippocampal ACh release and locomotor activity (Toide, 1989; Day et al., 1991) via the activation of nicotinic ACh receptors (O'Neill et al., 1994), and the circadian locomotion rhythm was weakened in rats whose ACh-producing neurons projecting to the hippocampus were destroyed (Lehmann et al., 2002).

ACh release in the rat brain has been shown to be suppressed by general anesthetics, except for ketamine (Kikuchi et al., 1997, 1998; Shichino et al., 1998). In these studies, however, the observational periods were limited to the period during and immediately after the administration of anesthetics. To date, there is increasing evidence that the influence of anesthetics on the brain remains after their pharmacological elimination from the body (Stratmann et al., 2009; Lin and Zuo, 2011).

We hypothesized that isoflurane anesthesia influences the circadian rhythm of body function in the same way as propofol anesthesia does (Challet et al., 2007; Dispersyn et al., 2008; Dispersyn et al., 2009a). The aims of our study were thus: (1) to investigate the effects of isoflurane anesthesia on the circadian rhythms of hippocampal extracellular ACh levels and rest–activity in rats, and (2) to study the impact of the circadian phase (light or dark phase) in which anesthesia was administered. No previous studies have been conducted on the long-lasting effects of volatile anesthetics on the circadian rhythms of neurotransmitter release.

EXPERIMENTAL PROCEDURES

Animals and surgery

All experiments conformed to the international guidelines on the ethical use of animals (Institute of Laboratory Animal Research and on Life Sciences, 1996), and all efforts were made to minimize pain or discomfort to the animals. Animal housing and surgical procedures were approved by the Institutional Animal Care and Use Committee of Yokohama City University Graduate School of Medicine (Approval No. 07-93).

Male Wistar-Imamichi rats (weighing 230–350 g) were purchased from Animal Reproduction Research Co. (Omiya, Japan). The rats were housed at a constant temperature (23 ± 2 °C) under a light/dark cycle (lights on: 0700–1900, 250 lux) in soundproof experimental booths. Food and water were available *ad libitum* throughout the experimental period.

Animals were administered intraperitoneal anesthesia with sodium pentobarbital (31.5 mg/kg) and local lidocaine (1%; Astra-Zeneca K.K., Osaka, Japan) infiltration; a stainless steel guide cannula (AG-8; Eicom Co., Kyoto, Japan) was implanted stereotactically into the dorsal hippocampus, as described previously (Kikuchi et al., 1998). Each rat was individually housed in a cylindrical plastic cage (diameter, 35 cm; height, 45 cm) with a sufficient amount of wood chips for 3-cm thickness, and free access to food and water for 1 week before the experiments to allow recovery after surgery and acclimatization. *In-vivo* microdialysis and measurement of locomotor activity, and isoflurane anesthesia were performed in the cylindrical plastic cage covered with food wrap on the upper portion in order to prevent anesthetic gas from diffusing in the experimental environment. The gas including anesthetics was passively exhausted from the top of the cylindrical cage to an excess anesthetic gas discharge device.

Isoflurane anesthesia

The rats were divided into three groups (n = 6 in each group): (1) the control group, to which neither carrier gas nor isoflurane was administered throughout the experimental period; (2) the dark-phase anesthesia group (DA group), to which 1.4% isoflurane was administered for 4 h during the middle of the first dark phase (2300–0300); and (3) the light-phase anesthesia group (LA group), to which 1.4% isoflurane was administered for 4 h during the middle of the first light phase (1100–1500).

The experiments were performed throughout in the cylindrical plastic cage. Isoflurane anesthesia was administered with a total flow of 6 L/min of carrier gas (60% nitrogen, 40% oxygen), which was introduced into the upper portion of the plastic cage. This 1.4% isoflurane was considered to be a 1.0 minimum alveolar concentration (Quasha et al., 1980; de Wolff et al., 1999). Isoflurane, carbon dioxide, and oxygen concentrations were monitored at the bottom of the plastic cage during anesthesia by using an anesthetic gas monitor (UIT-S-31-02; Datex Instrumentarium Co., Helsinki, Finland). We confirmed that, under these conditions, the isoflurane concentration reached a plateau within 10 min in all cases. In control group, we preliminarily compared locomotor activity with or without carrier gas flow (n = 2 for each situation) for 72 h. In aspect of locomotor activity, carrier gas flow did not affect the diurnal rhythm of locomotor activity. So we collected the data without carrier gas flow in control group.

In-vivo microdialysis and determination of ACh and locomotor activity

Five hours before starting experiment, the rats were lightly-sedated with isoflurane using an anesthetic jar, and the stylet was replaced with a microdialysis probe (AI-8-1; Eicom Co.) (Kikuchi et al., 1997; Kikuchi et al., 1998). This procedure took less than 2 min. Artificial cerebrospinal fluid (aCSF) (147 mM NaCl, 4 mM KCl, 1.2 mM $CaCl_2$, and 0.9 mM $MgCl_2$) was perfused through the dialysis probe with a 1.0-mm-long semi-permeable membrane (1.2 μL/min) using a microdialysis pump under unrestrained conditions. Dialysates were automatically collected in an autoinjector for 20 min and mixed with the same volume of ethylhomocholine solution (100 nM; internal standard). This mixture was then injected directly into a high-performance liquid chromatography system (HTEC-500; Eicom Co.) every 20 min. ACh was quantified by a combination of high-performance liquid chromatography, enzyme assay, and electrochemical detection. Details of the ACh assay procedure were described previously (Mizuno et al., 1991; Kikuchi et al., 1998), except that physostigmine was not used in the present study (Mitsushima et al., 2009). Before starting data collection,