Anesthesiology 2005; 102:1158–64 © 2005 American Society of Anesthesiologists, Inc. Lippincott Williams & Wilkins, Inc.

Effect of Sleep Deprivation on Righting Reflex in the Rat Is Partially Reversed by Administration of Adenosine A1 and A2 Receptor Antagonists

Avery Tung, M.D., Stacy Herrera, B.A.,† Martin J. Szafran, B.S.,† Kristen Kasza, M.A.,‡ Wallace B. Mendelson, M.D.§*

Background: **Similarities between naturally occurring sleep and general anesthesia suggest that the two states may interact physiologically. The authors have previously demonstrated that sleep deprivation potentiates anesthetic-induced loss of righting reflex (LORR) in rats. One possible mediator for this effect is adenosine, which accumulates in the brains of sleep-deprived animals and reduces anesthetic requirements. The authors tested in rats the hypothesis that potentiating effects of sleep deprivation on LORR can be altered by adenosine A1 and A2a receptor antagonists.**

Methods: **Five experiments were conducted. In each, rats underwent four trials, consisting of a 24-h period of either sleep deprivation or *ad libitum* activity followed by administration of a fixed dose of an adenosine antagonist or vehicle. Rats were then given isoflurane, and the time to LORR and recovery were measured. Each experiment tested a specific dose of an A1 receptor antagonist (8-cyclopentyltheophylline given *via* microinjection into the basal forebrain), an A2a receptor antagonist (ZM241385 *via* intraperitoneal administration), or both. In each experiment, all rats received all combinations of activity and drug/vehicle, separated by 5–7 days.**

Results: **In rested rats, neither antagonist altered the time to LORR. In sleep-deprived rats, both ZM241385 and 8-cyclopentyltheophylline prolonged the time to LORR and shortened recovery in a dose-dependent manner. Prolongation also occurred when subtherapeutic doses of both agents were coadministered.**

Conclusion: **Both antagonists partially reversed the effect of sleep deprivation on anesthetic action. This result implies that deprivation-induced changes in adenosine receptor activity can alter LORR. Neither antagonist completely reversed this effect, suggesting possible non–adenosine-mediated effects of sleep deprivation.**

UNLIKE anesthesia, naturally occurring sleep is endogenously generated, readily reversible with external stimuli, and characterized by discrete patterns of electroencephalographic/electromyographic activity.[1] Both sleep and general anesthesia, however, decrease responsiveness to external stimuli. Moreover, electrical and metabolic similarities in brain activity during both sleep and anesthesia[2,3] and an ability of anesthetic agents to induce sleep[4] have been observed. These findings have raised the possibility that regulatory interactions between sleep and general anesthesia exist and imply that some characteristics of general anesthesia may be generated by neuronal networks normally involved in producing the sleep state.[5]

We have previously demonstrated that sleep deprivation significantly enhances anesthetic-induced loss of righting reflex (LORR) in rats.[6] Although the molecular mechanisms that mediate this effect are incompletely understood, sleep deprivation induces an increase in brain adenosine concentrations in animals.[7] In addition, antagonist studies have suggested that adenosine increases sleep propensity in the basal forebrain *via* A1 receptors[8] and in the subarachnoid space *via* A2a receptors.[9] Along with the observation that exogenously administered adenosine enhances anesthetic potency,[10] these results suggest that changes in endogenous adenosine levels due to sleep deprivation may act *via* both A1 and A2a mechanisms and at different locations to mediate effects of sleep deprivation on anesthetic action.

If deprivation-induced changes in adenosine receptor activity represent a link between sleep loss and anesthetic action, pharmacologic modulation of adenosine receptor function may alter the effects of sleep deprivation on anesthetic potency. To test this hypothesis, we administered adenosine A1 and A2a receptor antagonists to rested and sleep-deprived rats. We first subjected rats to either *ad libitum* activity or sleep deprivation. In separate experiments, we then administered the adenosine A1 receptor antagonist 8-cyclopentyltheophylline (8-CPT) into the basal forebrain by direct microinjection, the adenosine A2a receptor antagonist ZM241385 by intraperitoneal injection, or both. We then exposed rats to isoflurane and compared the time to loss of spontaneous movement and righting reflex with antagonist to that in rats given vehicle. We hypothesized that if the ability of sleep deprivation to accelerate anesthetic-induced LORR was mediated by adenosine, such an effect would be modulated by blockade of brain adenosine receptors.

Materials and Methods

This study was performed with approval from the Institutional Animal Care and Use Committee at our institution (University of Chicago, Chicago, Illinois). Male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) weighing 250–300 g were anesthetized with

* Associate Professor of Anesthesia and Critical Care, † Research Technician, Department of Anesthesia and Critical Care, ‡ Biostatistician, Department of Biostatistics, § Professor of Psychiatry and Clinical Pharmacology, Department of Psychiatry.

Received from the Departments of Anesthesia and Critical Care, Biostatistics, and Psychiatry, The University of Chicago, Chicago, Illinois. Submitted for publication August 19, 2004. Accepted for publication February 16, 2005. Supported by grant No. K08-GM000697 (to Dr. Tung) from the National Institutes of Health, Bethesda, Maryland, and The University of Chicago Department of Anesthesia and Critical Care, Chicago, Illinois. Presented in part at the Annual Meeting of the American Society of Anesthesiologists, Orlando, Florida, October 15, 2002.

Address reprint requests to Dr. Tung: Department of Anesthesia and Critical Care, The University of Chicago, 5841 S. Maryland Ave. MC4028, Chicago, Illinois 60637. Address electronic mail to: atung@airway.uchicago.edu. Individual article reprints may be purchased through the Journal Web site, www.anesthesiology.org.

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/102/6/1158/358494/0000542-200506000-00015.pdf by guest on 09 April 2024

intraperitoneal ketamine (70 mg/kg) and xylazine (6 mg/kg). Five stainless steel screws (Small Parts Inc., Miami Lakes, FL) were implanted through the skull to serve as dural electroencephalographic electrodes, and two electromyographic electrodes were implanted in the neck musculature. In rats scheduled to undergo intracranial microinjection of 8-CPT, bilateral 24-gauge stainless steel guide cannulae were then placed into the basal forebrain using stereotactic coordinates derived from Paxinos and Watson[11] and our earlier work: anterior/posterior −0.4, medial/lateral ±2.0, dorsal/ventral +8.0 mm from bregma. At the conclusion of surgery, a 31-gauge stainless steel stylet was placed into each guide cannula to maintain patency. All rats were then allowed to recover for 7 days in a temperature (21°–24°C)– and light-controlled room with *ad libitum* access to food and water. Lights were turned on at 6:00 AM and off at 6:00 PM every day.

Five separate experiments were performed in five separate groups of rats. In each group, rats underwent four trials, consisting of all combinations of a behavioral intervention (24-h period of *ad libitum* activity or total sleep deprivation beginning and ending at 12:00 noon), followed by administration of a specific dose of adenosine antagonist(s) or vehicle. The five groups differed on the basis of the dose and drug administered:

Group 1 (n = 12): microinjection of low-dose 8-CPT (0.25 ng)
Group 2 (n = 12): microinjection of high-dose 8-CPT (0.50 ng)
Group 3 (n = 15): intraperitoneal administration of low-dose ZM241385 (5 mg/kg)
Group 4 (n = 15): intraperitoneal administration of high-dose ZM241385 (10 mg/kg)
Group 5 (n = 10): combined administration of low-dose 8-CPT and ZM241385 (0.25 ng and 5 mg/kg, respectively)

Sleep deprivation was achieved by the disk-over-water method.[12] Specifically, rats were placed on a 45-cm-diameter disk suspended over a pan of water with continuous computerized electroencephalographic and electromyographic monitoring. When sleep onset was detected, the computer rotated the disk at a rate of 3 revolutions/min, causing the rat to wake up and walk to avoid falling into the water. When the rat awakened, rotation stopped. This method has previously been validated as able to produce near-total sleep deprivation without excessive physical exertion.[13] Rats receiving *ad libitum* activity were exposed to the same environment, except that a platform was placed over the wheel to eliminate the water hazard and effects of wheel rotation.

Immediately after the behavioral intervention (12:00 noon), rats were given either an adenosine antagonist or vehicle. In the first two groups (1 and 2), the adenosine A1 antagonist 8-CPT was microinjected into the basal forebrain. After removal of the stylet from the guide cannula implanted during surgery, a 31-gauge stainless steel injection cannula (Plastics One, Roanoke, VA) was inserted so that the tip extended 1.0 mm past the tip of the guide cannula. After warming to 37°C, 8-CPT (Sigma Chemicals, St. Louis, MO) dissolved in 0.2 μl artificial cerebrospinal fluid (145 mM Na^+, 1.2 mM Ca^{2+}, 2.7 mM K^+, 1.0 mM Mg^{2+}, and 150 mM Cl^-, pH 7.4), or artificial cerebrospinal fluid alone was injected *via* a 10-μl Hamilton syringe through PE-20 tubing attached to the injection cannula. The injection was made over 30 s, and the inner cannula left in place for 30 s after each injection. Each rat received bilateral injections for a total volume of 0.4 μl. Volumes and infusion rates were calculated to minimize tissue damage and limit diffusion of drug from the injection site.[14] Each group received a different dose of 8-CPT. The first group received a 2.5-μM solution (0.25 ng total in 0.4 μl), and the second group received a 5-μM solution (0.5 ng total in 0.4 μl). After injection, rats were returned to their cages for a 15-min incubation period to allow drug diffusion, and those undergoing sleep deprivation were kept awake by gently shaking the cage when sleep was observed.

In the second two groups (3 and 4), the adenosine A2a antagonist ZM241385 was administered. ZM241385 (Sigma Chemicals) was dissolved in a 1:1 mixture of Alkamuls EL-620 (Rhone-Poulenc, Collegeville, PA) and dimethyl sulfoxide (Fisher Scientific, Fair Lawn, NJ). The resulting mixture was then diluted 1:4 in 0.9 normal saline. Because this mixture was too viscous to be administered *via* microinjection, it was administered *via* intraperitoneal injection. One hour before the end of the behavioral intervention (11:00 AM), rats were given either ZM241385 or the Alkamuls–dimethyl sulfoxide–saline vehicle. The higher dose of ZM241385 was 10 mg/kg body weight, and the lower dose was 5 mg/kg. The final injection volume for all rats was 2 ml. Rats were then returned to their cages after injection, and those undergoing sleep deprivation were kept awake by gently shaking the cage.

In the fifth group, rats received both antagonists together or both vehicles. Forty-five minutes before the end of the behavioral intervention, rats were given ZM241385 (5 mg/kg) or vehicle *via* intraperitoneal injection. Immediately after the end of deprivation, rats were then given 8-CPT (0.25 ng) or vehicle *via* bilateral microinjection into the basal forebrain. Rats were then returned to their cages for an additional 15-min incubation period, and those undergoing sleep deprivation were kept awake by gently shaking the cage.

After drug administration and incubation, rats were given isoflurane, and the time to LORR/spontaneous movement and recovery were measured as follows. Rats were placed in a 13 × 13 × 23-cm clear plastic anesthesia chamber fitted with a gas inlet and outlet. A compressed oxygen tank and a standard isoflurane vaporizer

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/102/6/1158/358494/0000542-200506000-00015.pdf by guest on 09 April 2024

(Cyprane Ltd., Keighley, England, United Kingdom) were used to direct a constant 3-l/min flow of oxygen containing 1.1% isoflurane (Abbott Laboratories, Chicago, IL) through the box. The isoflurane concentration was chosen to allow detection of accelerated LORR in sleep-deprived rats.[6] Isoflurane concentrations were monitored continuously using a Puritan-Bennett Datex model 254 airway gas monitor (Puritan Bennett, Carlsbad, CA) calibrated before each use with a reference gas containing 1.5% isoflurane (Biochem Int., Waukesha, WI). The presence or absence of righting reflex and attempts to self-right were tested by rotating the box every 10 s (or after cessation of spontaneous movement) and observing the rat to verify that no righting attempts or movements in response to repeated positioning on their back or side were observed. When loss of all attempts to self-right (despite repositioning or box rotation) was achieved, rats were removed from the chamber and allowed to recover while spontaneously breathing room air. Rats were gently prodded every 15 s to determine the onset of recovery, defined as the return of righting reflex. An investigator blinded to the deprivation and antagonist history of the rat recorded the time from initial exposure to isoflurane to LORR and from discontinuation of the anesthetic to recovery.

After recovery, rats were returned to their temperature- and light-controlled colony. After a 5- to 7-day recovery period, the above protocol was repeated in random order until each rat had received every possible combination of behavioral intervention and drug (sleep deprivation and vehicle, sleep deprivation and antagonist, *ad libitum* activity and vehicle, *ad libitum* activity and antagonist). When all treatments had been completed, rats were killed *via* intraperitoneal injection of 400 mg/kg sodium pentobarbital. Those with intracranial cannulae were perfused transcardially with 0.9% NaCl followed by 4% formalin in 1.25% NaCl. After perfusion, rats were decapitated, and the brain was extracted and stored in a solution of 4% formalin–30% sucrose for 48 h. Coronal brain sections (40 μm) were cut on a freezing microtome, mounted, and stained with cresyl violet. The tip of the injection cannula track was then localized by light microscopy.

Electroencephalographic/electromyographic data were recorded on a Grass model 78 polygraph (Grass-Telefactor, West Warwick, RI) and relayed to a computer for digital recording. Data were divided into 30-s epochs and scored as waking, nonrapid eye movement, or rapid eye movement sleep using an automated scoring system previously validated against visual and behavioral methods.[12,13] Portions of the electroencephalogram/electromyogram were also scored visually to verify the reliability of the automated system. Definitions of sleep stages have been presented in detail previously.[15]

Statistical Analysis

We performed five experiments in five separate groups of rats, testing two doses of 8-CPT, two doses of ZM241385, and one dose of both antagonists given together. Rats in each group served as their own controls, receiving all four combinations of sleep deprivation/*ad libitum* activity and drug/placebo. Data collected included weights; ages; rapid eye movement, nonrapid eye movement, and total sleep times for rats during sleep deprivation; and cumulative duration of wheel rotation for all groups. The times to LORR and to recovery were recorded by an observer blinded to the deprivation and microinjection history of the rat. All values were expressed as mean ± SD. Repeated-measures analysis of variance was used to determine the significance of our observed outcomes, treating condition (*ad libitum vs.* sleep deprivation), and treatment (placebo *vs.* drug) as repeated factors for both drugs (8-CPT and ZM241385) and both doses (high and low). *Post hoc* comparisons were made using the Bonferroni adjustment. Random-effects mixed models were used to examine the effect of age and weight on time to LORR and to recovery. All analyses were performed using Stata version 8 (Stata Corp., College Station, TX).

Results

Although completion of the four trials required as many as 21 days for some rats, average weights, ages, amount of wheel rotation, and sleep frequencies were similar within and between all groups. For all rats, the wheel rotated between 14 and 20% of the time, resulting in approximately 90% wakefulness during the 24-h behavioral intervention. Analysis of variance revealed no significant effect of weight, age, or sequence of treatments on the amount of sleep during deprivation or the amount of wheel rotation. For rats with intracranial cannulae, the average location of the 8-CPT injection sites in millimeters was as follows: (mean ± SD) anterior/posterior −0.32 ± 0.06, medial/lateral 1.9 ± 0.42 (L), 2.0 ± 0.36 (R), dorsal/ventral 8.6 ± 0.25 (L), 8.7 ± 0.24 (R). Figure 1 depicts the location of individual injection sites.

In all experiments, rats given vehicle after 24 h of sleep deprivation lost their righting reflex with isoflurane significantly sooner than after the same duration of *ad libitum* activity (figs. 2–4). The time to recovery was also prolonged. This effect of sleep deprivation on LORR in untreated rats was similar in magnitude to findings reported previously.[6]

In rats given 8-CPT only, no difference in time to LORR or to recovery was seen in rested animals that received vehicle, low (0.25 ng) doses of 8-CPT, or high (0.5 ng) doses of 8-CPT (fig. 2). In sleep-deprived rats, basal forebrain microinjection of 8-CPT significantly delayed isoflurane-induced LORR in a dose-dependent manner.

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/102/6/1158/358494/0000542-200506000-00015.pdf by guest on 09 April 2024

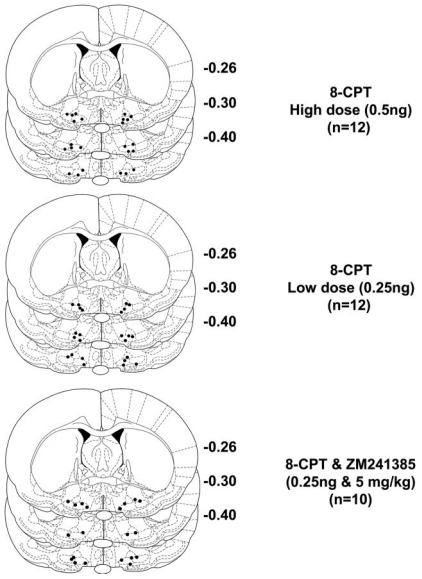

Fig. 1. Histologic localization of injection sites in rats receiving 8-cyclpentyltheophylline (8-CPT). Coronal brain slices are from Paxinos and Watson,[11] and numbers refer to distance in millimeters from bregma, where (−) = caudal.

Rats receiving the lower dose of 8-CPT demonstrated a slight, nonsignificant increase in time to LORR when compared with those receiving the artificial cerebrospinal fluid vehicle. At the higher dose, 8-CPT significantly increased the time to LORR (426.5 ± 92 *vs.* 264.8 ± 53 s [mean ± SD]; $F_{1,33} = 23.16$, $P = 0.001$; fig. 2). Time to recovery was also reduced in a dose-dependent manner, with the difference becoming significant at the higher dose (74.6 ± 18 *vs.* 115.0 ± 17 s; $F_{1,33} = 22.03$, $P <$ 0.02; fig. 2). Sleep-deprived rats treated with the high dose of 8-CPT still lost their righting reflex sooner than rested rats receiving vehicle (426.5 ± 92 *vs.* 693.4 ± 112 s; $F_{1,33} = 63.14$, $P = 0.0001$). Figure 2 represents a composite of low- and high-dose 8-CPT groups, where vehicle data from both groups are averaged together.

In rats given ZM241385 only, rested rats receiving

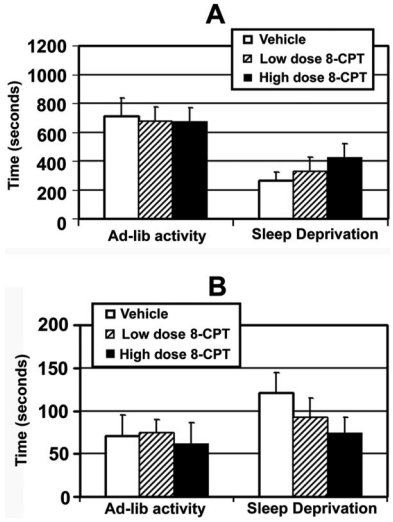

Fig. 2. Time to loss of righting reflex (*A*) and to recovery (*B*) in rats subjected to sleep deprivation or *ad libitum* activity followed by microinjection of 8-cyclopentyltheophylline (8-CPT) or vehicle. The *graph* depicts composite results from two separate experiments involving two doses of 8-CPT. The *vehicle bar* depicts the average of vehicle data from both high- and low-dose groups. High Dose = 0.5 ng total microinjection; Low Dose = 0.25 ng total microinjection. Values are expressed as mean ± SD. * $P < 0.05$ by repeated-measures analysis of variance.

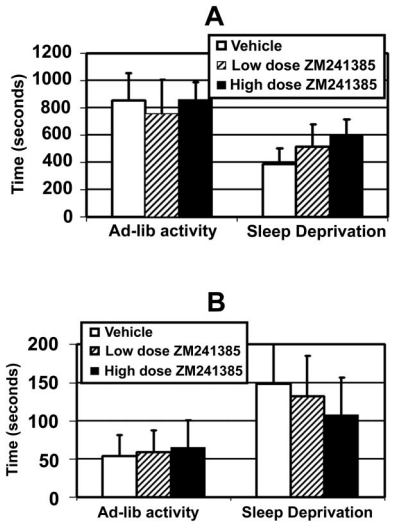

Fig. 3. Time to loss of righting reflex (*A*) and to recovery (*B*) in rats subjected to sleep deprivation or *ad libitum* activity followed by intraperitoneal administration of ZM241385 or vehicle. The *graph* depicts composite results from two separate experiments involving two doses of ZM241385. The *vehicle bar* depicts the average of vehicle data from both high- and low-dose groups. High Dose = 10 mg/kg intraperitoneal injection; Low Dose = 5 mg/kg intraperitoneal injection. Values are expressed as mean ± SD. * $P < 0.05$ by repeated-measures analysis of variance.

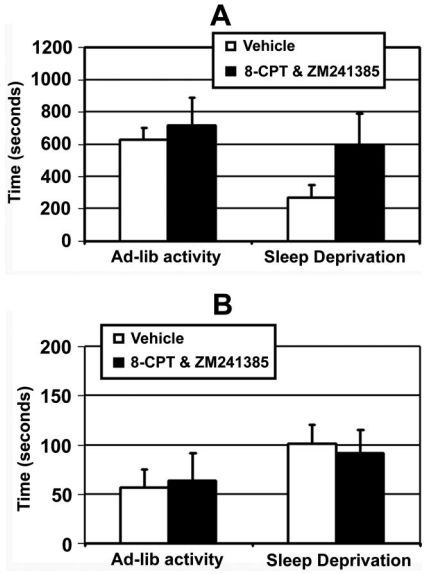

Fig. 4. Time to loss of righting reflex (*A*) and to recovery (*B*) in rats subjected to sleep deprivation or *ad libitum* activity followed by coadministration of both ZM241385 and 8-cyclopentyltheophylline (8-CPT) (*vs.* vehicle). Values are expressed as mean ± SD. * $P < 0.05$ by repeated-measures analysis of variance.

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/102/6/1158/358494/0000542-200506000-00015.pdf by guest on 09 April 2024

vehicle, low (5 mg/kg) doses, or high (10 mg/kg) doses also demonstrated no differences in time to LORR or recovery (fig. 3). As with 8-CPT, ZM241385 prolonged the time to LORR in a dose-dependent manner, reaching significance at the higher dose (596 ± 114 *vs.* 340.6 ± 76 s; $F_{1,42} = 29.46$, $P = 0.001$; fig. 3). Time to recovery was also significantly reduced at the higher dose (107.7 ± 50 *vs.* 157.3 ± 52 s; $F_{1,42} = 13.72$, $P = 0.0012$; fig. 3). As with rats receiving 8-CPT, deprived rats receiving the higher dose of ZM241385 lost their righting reflex sooner than rested rats (596 ± 114 *vs.* 892.1 ± 176.2 s; $F_{1,42} = 39.42$, $P = 0.0001$). Figure 3 represents a composite of low and high dose ZM241385 groups, where vehicle data from both groups are averaged together.

In rats given both 8-CPT and ZM241385, no effect of antagonist administration in rested rats was seen (fig. 4). In sleep-deprived rats, coadministration of 8-CPT and ZM241385 significantly prolonged the time to LORR over rats that received vehicle only (591 ± 201.8 *vs.* 267.9 ± 82.6 s; $F_{1,27} = 29.19$, $P < 0.033$; fig. 4). Although time to recovery was slightly lower in rats that received antagonist, the difference was not significant.

Discussion

Observations that anesthetic requirements vary with time of day[16] and that sleep deprivation enhances some measures of anesthetic potency[6] suggest that mechanisms involved in the homeostatic regulation of sleep may interact with and potentially modify the anesthetized state. In this article, we report that blockade of both adenosine A1 and A2a receptors in the rat partially prevented sleep deprivation from potentiating isoflurane-induced LORR. Specifically, we found in sleep-deprived rats that basal forebrain microinjection of the adenosine A1 receptor antagonist 8-CPT increased the time to LORR with isoflurane and reduced the time to recovery in a dose-dependent manner (fig. 1). Systemic administration of the adenosine A2a receptor antagonist ZM241385 had a similar effect (fig. 2). When the two antagonists were administered together in doses too low to have a significant effect separately, a similar prolongation of time to LORR was noted (fig. 3). These findings suggest that modulation of both adenosine A1 and A2a receptors partly uncouple the link between sleep homeostatic mechanisms and anesthetic potency and raise the possibility that adenosine may mediate such a link.

Our observations are supported by existing human and animal data. In humans, systemic administration of adenosine potentiates hypnosis induced by intravenous anesthetics[10] and reduces intraoperative anesthetic requirements.[17] In rats and cats, extracellular adenosine concentrations in the basal forebrain increase progressively with prolonged sleep loss and return to baseline levels with recovery sleep.[18] These changes in endogenous adenosine concentration also alter sleep behavior. Administration of exogenous adenosine reuptake inhibitors (or adenosine itself) into the basal forebrain increases sleep, whereas the adenosine A1 receptor antagonist 8-CPT produces the opposite effect.[18]

Although how the brain generates and maintains either the sleep or waking state is incompletely understood, adenosine may modulate the arousal state by altering interactions between specific neuronal cell groups active during wakefulness such as the basal forebrain (wake-active), and sleep-active cell groups such as the ventrolateral preoptic nucleus (VLPO).[8] Projections from the VLPO to the basal forebrain have led to hypotheses that sleep-active cell groups, when activated, may exert their effects by inhibiting the activity of wake-active neurons.[19] In an *in vitro* model, for example, adenosine administration to the VLPO acts on γ-aminobutyric acid–mediated synaptic inputs to "disinhibit" VLPO sleep-active neurons.[20] Behaviorally, sleep-inducing effects have been observed when adenosine is administered *via* microdialysis both to sleep-active and wake-active cell groups.[18,21]

Existing data also support a relation between increased adenosine concentrations and changes in anesthetic potency. Basal forebrain administration of the adenosine A1 receptor antagonist 8-CPT increases the discharge rate of cholinergic neurons known to be involved in arousal.[22] This effect of adenosine on brain cholinergic activity may plausibly allow it to modify anesthetic action. Direct administration of cholinergic agents into the pontine reticular nucleus, for example, inhibits spinal motoneuron excitability in cats[23] and reduces halothane minimum alveolar concentration (MAC) in rats.[24] In addition, halothane decreases pontine acetylcholine release,[25] and pontine administration of an adenosine A1 agonist both decreases acetylcholine release and alters recovery from halothane anesthesia.[26] Adenosine-induced modulation of brain cholinergic activity may thus have partly accounted for our observations.

Our finding that both adenosine A1 and A2a receptor antagonists blocked the effect of sleep deprivation on anesthetic potency suggests an effect mediated by both receptor subtypes. This possibility is consistent with previous reports implicating both receptors in the sleep-promoting effects of adenosine. In cats, delivery of the A1 receptor agonist N6-cyclo-hexyl-adenosine to the basal forebrain depressed the activity of wake-active neurons, whereas the A1 antagonist 8-CPT had the opposite effect, and the A2a agonist CGS-16284 had no effect.[8] In rats, increases in adenosine A1 and decreases in A2a receptor messenger RNA with sleep deprivation have been observed.[27] These results suggest that in the basal forebrain, adenosine acts *via* an A1 receptor–specific mechanism on sleep homeostasis.

Adenosine A2 receptors, however, have also been as-

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/102/6/1158/358494/0000542-200506000-00015.pdf by guest on 09 April 2024

sociated with sleep regulation. Existing data demonstrate in rats that subarachnoid administration of the adenosine A2a receptor antagonist KF17837 blocks the sleep-inducing effects of prostaglandin D2 and that direct microinjection of the adenosine A2a receptor agonist CGS-21680 into the ventrolateral preoptic area (VLPO) increases sleep.[9,28] Furthermore, although administration of both A1 and A2a agonists into the pontine reticular formation of the rat increases sleep, only the A2a effect is inhibited by atropine.[29] These data suggest a specific A2a effect, likely localized apart from the A1 site on the ventral surface of the rostral basal forebrain.[9]

Our rationale for administering A1 and A2a antagonists *via* different routes was thus based in part on the strong possibility that effects of adenosine on sleep homeostasis were likely to occur *via* different receptors and at different sites. In addition, we were unable to reliably administer ZM241385 *via* microinjection because of the viscosity of the Alkamuls–dimethyl sulfoxide–saline vehicle. Our finding that simultaneous administration of both agents in doses too low to be effective alone significantly prolonged time to LORR further suggests that both receptor subtypes have important roles in the effect of adenosine on sleep deprivation and may interact physiologically in their effects on anesthetic potency as well.

We were only able to partially reverse the effect of sleep deprivation on LORR. At the higher dose of either antagonist, sleep-deprived rats continued to lose their righting reflex sooner than if they had not been deprived. Several possible reasons exist for this incomplete reversal. First, the doses of antagonists that we used, although physiologically significant, may not have been adequate. Second, neither antagonist would have blocked adenosine effects on other receptor subtypes. 8-CPT may have blocked A1 effects of adenosine, for example, but would not have prevented A2a receptor effects. Third, our incubation periods (12–15 min for 8-CPT, 60 min for ZM241385) may not have captured the peak effect of our intervention. Finally, effects of sleep deprivation unrelated to changes in adenosine receptor activity may also modulate anesthetic potency. The endogenous sleep modulator oleic acid amide, for example, not only accumulates in the cerebrospinal fluid of sleep-deprived rats and cats, but also enhances γ-aminobutyric acid type A activity *in vitro*[30] and potentiates the activity of benzodiazepines.[31]

Our study has several important limitations. First, we did not measure adenosine concentrations and can only infer the relation among sleep deprivation, increased adenosine concentrations, and anesthetic action that our data suggest. It is possible, for example, that sleep deprivation alters the transduction of the adenosine signal downstream from the adenosine receptor, or the number of adenosine receptors in addition to adenosine levels. Clarifying this issue would require the measurement of adenosine concentrations during sleep deprivation, recovery sleep, and general anesthesia. Second, it is possible that both adenosine antagonists induced a nonspecific, general increase in resistance to anesthetic action. We believed such a possibility was unlikely, however, because we observed no effect in rested rats (when compared to vehicle) at the doses we tested. Third, our study design did not measure anesthetic effects at steady state. Although it was therefore possible that we were measuring a pharmacokinetic and not a pharmacodynamic effect of adenosine antagonism on anesthetic potency, we thought it was unlikely, given the magnitude of the pharmacokinetic change that would have had to occur to produce our results.

We did not use MAC as our endpoint for anesthetic action for several reasons. First, determination of MAC can require a prolonged period of anesthetic titration. This may have caused us to miss peak levels of drug effect. Because rats may discharge accumulated sleep debt while anesthetized,[32] the duration of anesthesia required to determine MAC may have introduced variability in the "dose" of sleep deprivation. Also, MAC involves a painful stimulus, which may be perceived differently in sleep-deprived subjects.[33] In addition, MAC is unchanged by spinal cord transaction in rats[34] and thus may represent effects of anesthetics primarily on spinal cord reflex pathways. Although successful righting is in part also spinally mediated, righting attempts occur despite transections as high as the postcollicular level.[35]

Our study involved a rat model of sleep homeostasis. Although this model has been used extensively to model human sleep behavior, differences between rats and humans in the behavioral response to sleep deprivation or anesthesia may limit ready generalization of our results. Nevertheless, we believe that our data support a potential relation between preoperative sleep adequacy and perioperative anesthetic management. Both anecdotal reports[36] and clinical trials[37] link sleep apnea to enhanced respiratory depressant effects of sedatives. Such clinical reports suggest an important possible role for sleep deprivation in potentiating sensitivity to anesthetic drugs and underscore the importance of diagnosing sleep apnea in the perioperative period. Because a 24-h period of sleep deprivation in the rat cannot be correlated to an equivalent duration of sleeplessness in humans, it is not possible to extrapolate how much sleep deprivation is required to clinically alter anesthetic action. However, reducing sleep to less than 5 h in children undergoing noninvasive procedures with conscious sedation significantly increased periprocedure nursing care requirements.[38] Studies such as these suggest that sleep deprivation in humans can adversely affect anesthetic care and perioperative management.

In conclusion, we report an ability of both the adeno-

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/102/6/1158/358494/0000542-200506000-00015.pdf by guest on 09 April 2024

sine A1 receptor antagonist 8-CPT and the A2a receptor antagonist ZM241385 to partially reverse the effect of sleep deprivation on isoflurane-induced LORR in the rat. Although neither antagonist altered LORR in rested rats, both increased the time to isoflurane-induced LORR in sleep-deprived rats and shortened the time to recovery. Our results suggest that one possible link between sleep deprivation and changes in anesthetic potency may be fluctuations in regional adenosine concentrations induced by sleep deprivation. These findings support the hypothesis that neuronal networks active during sleep may have a significant role in generating the reduced responsiveness characteristic of general anesthesia. Further elucidating the relation between sleep homeostatic regulatory mechanisms and anesthetic action may help to clarify the mechanisms of general anesthesia and may allow anesthesiologists to understand some of the sources of variability in anesthetic effect.

References

1. Carskadon MA, Dement WC: Normal human sleep: An overview, Principles and Practice of Sleep Medicine, 3rd edition. Edited by Kryger MH, Roth T, Dement WC. Philadelphia, WB Saunders, 2000, pp 15–25
2. Sleigh JW, Andrzejowski J, Steyn-Ross A, Steyn-Ross M: The bispectral index: A measure of depth of sleep? Anesth Analg 1999; 88:659–61
3. Alkire MT, Pomfrett CJ, Haier RJ, Gianzero MV, Chan CM, Jacobsen BP, Fallon JH: Functional brain imaging during anesthesia in humans: Effects of halothane on global and regional cerebral glucose metabolism. ANESTHESIOLOGY 1999; 90:701–9
4. Tung A, Bluhm B, Mendelson WB: The hypnotic effect of propofol in the medial preoptic area of the rat. Life Sci 2001; 69:855–62
5. Lydic R, Biebuyck J: Sleep neurobiology: Relevance for mechanistic studies of anaesthesia. Br J Anaesth 1994; 72:506–8
6. Tung A, Szafran MJ, Bluhm B, Mendelson WB: Sleep deprivation potentiates isoflurane and propofol-induced loss of righting reflex in the rat. ANESTHESIOLOGY 2002; 97:906–11
7. Porkka-Heiskanen T, Strecker RE, Thakkar M, Bjorkum AA, Greene RW, McCarley RW: Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. Science 1997; 276:1265–8
8. Thakkar MM, Delgiacco RA, Strecker RE, McCarley RW: Adenosinergic inhibition of basal forebrain wakefulness-active neurons: A simultaneous unit recording and microdialysis study in freely behaving cats. Neuroscience 2003; 122:1107–13
9. Satoh S, Matsumura H, Suzuki F, Hayaishi O: Promotion of sleep mediated by the A2a-adenosine receptor and possible involvement of this receptor in the sleep induced by prostaglandin D2 in rats. Proc Natl Acad Sci U S A 1996; 93:5980–4
10. Kaputlu I, Sadan G, Ozdem S: Exogenous adenosine potentiates hypnosis induced by intravenous anaesthetics. Anaesthesia 1998; 53:496–500
11. Paxinos G, Watson CR: AChE stained horizontal sections of the rat brain in stereotaxic coordinates. J Neurosci Methods 1980; 3:129–49
12. Rechtschaffen A, Bergmann BM: Sleep deprivation in the rat by the disk-over-water method. Behav Brain Res 1995; 69:55–63
13. Bergmann BM, Kushida CA, Everson CA, Gilliland MA, Obermeyer W, Rechtschaffen A: Sleep deprivation in the rat: II. Methodology. Sleep 1989; 12:5–12
14. Meyers RD: Injection of solutions into cerebral tissue: Relation between volume and diffusion. Physiol Behav 1966; 1:171–4
15. Bergmann BM, Winter JB, Rosenberg RS, Rechtschaffen A: NREM sleep with low-voltage EEG in the rat. Sleep 1987; 10:1–11
16. Munson ES, Martucci RW, Smith RE: Circadian variations in anesthetic requirement and toxicity in rats. ANESTHESIOLOGY 1970; 32:507–14
17. Seitz PA, ter Riet M, Rush W, Merrell WJ: Adenosine decreases the minimum alveolar concentration of halothane in dogs. ANESTHESIOLOGY 1990; 73:990–4
18. Strecker RE, Morairty S, Thakkar MM, Porkka-Heiskanen T, Basheer R, Dauphin LJ, Rainnie DG, Portas CM, Greene RW, McCarley RW: Adenosinergic modulation of basal forebrain and preoptic/anterior hypothalamic neuronal activity in the control of behavioral state. Behav Brain Res 2000; 115:183–204
19. Saper CB, Chou TC, Scammell TE: The sleep switch: Hypothalamic control of sleep and wakefulness. Trends Neurosci 2001; 24:726–31
20. Morairty S, Rainnie D, McCarley R, Greene R: Disinhibition of ventrolateral preoptic area sleep-active neurons by adenosine: A new mechanism for sleep promotion. Neuroscience 2004; 123:451–7
21. Scammell TE, Gerashchenko DY, Mochizuki T, McCarthy MT, Estabrooke IV, Sears CA, Saper CB, Urade Y, Hayaishi O: An adenosine A2a agonist increases sleep and induces Fos in ventrolateral preoptic neurons. Neuroscience 2001; 107:653–63
22. Noor Alam MD, Syzmusiak R, Gong H, King J, McGinty D: Adenosinergic modulation of rat basal forebrain neurons during sleep and waking: Neuronal recording with microdialysis. J Physiol 1999; 521:679–90
23. Morales FR, Engelhardt JK, Soja PJ, Pereda AE, Chase MH: Motoneuron properties during motor inhibition produced by microinjection of carbachol into the pontine reticular formation of the decerebrate cat. J Neurophysiol 1987; 57:1118–29
24. Ishizawa Y, Ma HC, Dohi S, Shimonaka H: Effects of cholinomimetic injections into the brain stem reticular formation on halothane anesthesia and antinociception in rats. J Pharmacol Exp Ther 2000; 293:845–51
25. Keifer JC, Baghdoyan HA, Becker L, Lydic R: Halothane decreases pontine acetylcholine release and increases EEG spindles. Neuroreport 1994; 5:577–80
26. Tanase D, Baghdoyan HA, Lydic R: Dialysis delivery of an adenosine A1 receptor agonist to the pontine reticular formation decreases acetylcholine release and increases anesthesia recovery time. ANESTHESIOLOGY 2003; 98:912–20
27. Basheer R, Halldner L, Alanko L, McCarley RW, Fredholm BB, Porkka-Heiskanen T: Opposite changes in adenosine A1 and A2A receptor mRNA in the rat following sleep deprivation. Neuroreport 2001; 12:1577–80
28. Scammell TE, Gerashchenko DY, Mochizuki T, McCarthy MT, Estabrooke IV, Sears CA, Saper CB, Urade Y, Hayaishi O: An adenosine A2a agonist increases sleep and induces Fos in ventrolateral preoptic neurons. Neuroscience 2001; 107:653–63
29. Marks GA, Shaffery JP, Speciale SG, Birabil CG: Enhancement of rapid eye movement sleep in the rat by actions at A1 and A2a adenosine receptor subtypes with a differential sensitivity to atropine. Neuroscience 2003; 116:913–20
30. Lees G, Edwards MD, Hassoni AA, Ganellin CR, Galanakis D: Modulation of GABA(A) receptors and inhibitory synaptic currents by the endogenous CNS sleep regulator cis-9,10-octadecenoamide (cOA). Br J Pharmacol 1998; 124:873–82
31. Yost CS, Hampson AJ, Leonoudakis D, Koblin DD, Bornheim LM, Gray AT: Oleamide potentiates benzodiazepine-sensitive gamma-aminobutyric acid receptor activity but does not alter minimum alveolar anesthetic concentration. Anesth Analg 1998; 86:1294–300
32. Tung A, Bergmann BM, Herrera S, Cao D, Mendelson WB: Recovery from sleep deprivation occurs during propofol anesthesia. ANESTHESIOLOGY 2004; 100:1419–26
33. Onen SH, Alloui A, Gross A, Eschallier A, Dubray C: The effects of total sleep deprivation, selective sleep interruption and sleep recovery on pain tolerance thresholds in healthy subjects. J Sleep Res. 2001; 10:35–42
34. Rampil IJ: Anesthetic potency is not altered after hypothermic spinal cord transaction in rats. ANESTHESIOLOGY 1994; 80:606–10
35. Bignall KE: Ontogeny of levels of neural organization: The righting reflex as a model. Exp Neurol 1974; 42:566–73
36. Ostermeier AM, Roizen MF, Hautkappe M, Klock PA, Klafta JM: Three sudden postoperative respiratory arrests associated with epidural opioids in patients with sleep apnea. Anesth Analg 1997; 85:452–60
37. Waters KA, McBrien F, Stewart P, Hinder M, Wharton S: Effects of OSA, inhalational anesthesia, and fentanyl on the airway and ventilation of children. J Appl Physiol 2002; 92:1987–94
38. Shields CH, Johnson S, Knoll J, Chess C, Goldberg D, Creamer K: Sleep deprivation for pediatric sedated procedures: Not worth the effort. Pediatrics 2004; 113:1204–8

Downloaded from http://asa2.silverchair.com/anesthesiology/article-pdf/102/6/1158/358494/0000542-200506000-00015.pdf by guest on 09 April 2024

Copyright © by the American Society of Anesthesiologists. Unauthorized reproduction of this article is prohibited.