Chronobiology International, 24(3): 463–470, (2007)

ISSN 0742-0528 print/1525-6073 online
DOI: 10.1080/07420520701420675

informa
healthcare

EVENING ALCOHOL SUPPRESSES SALIVARY MELATONIN IN YOUNG ADULTS

Tracy L. Rupp,[1] Christine Acebo,[1,2] and Mary A. Carskadon[1,2,3]

[1]*E.P. Bradley Sleep and Chronobiology Laboratory, Providence, Rhode Island, USA*
[2]*The Warren Alpert Medical School of Brown University, Providence, Rhode Island, USA*
[3]*Brown University Department of Psychology, Providence, Rhode Island, USA*

The study objective was to determine the acute effects of a moderate evening dose of alcohol on salivary melatonin levels in humans with stable prior sleep-wake histories and in a controlled environment. Twenty-nine adults (nine males) ages 21 to 25 ($M = 22.6$, $SD = 1.2$) yrs adhered to a 10-day at-home stabilized sleep schedule followed by three in-lab adaptation, placebo, and alcohol (order counterbalanced) study nights. Alcohol (vodka: 0.54 g/kg for men and 0.49 g/kg for women) or placebo beverage was consumed over 30 min, ending 1 h before stabilized bedtime. At 140 and 190 min after alcohol administration, melatonin level was reduced by 15% and 19%, respectively, in comparison to placebo. The findings indicate that a moderate dose of alcohol in the evening suppressed melatonin in young adults.

Keywords Alcohol, Melatonin suppression, Dim light

INTRODUCTION

Twenty-six percent of young adults in the United States turn to alcohol as a sleep aid (National Sleep Foundation, 2000), despite experimental evidence indicating that alcohol consumption increases sleep fragmentation and disruption. The mechanisms underlying the disruptive impact of alcohol on sleep are not entirely known; however, a few studies suggest that the suppression of melatonin by evening alcohol may be a contributing factor (Ekman et al., 1993; Rojdmark et al., 1993).

Submitted November 28, 2006, Returned for revision January 4, 2007, Accepted January 15, 2007

This research was supported by the National Institute on Alcohol Abuse and Alcoholism Grant AA13252 to Mary A. Carskadon.

Address correspondence to Tracy L. Rupp , Ph.D., Department of Behavioral Biology, Division of Psychiatry and Neuroscience, Walter Reed Army Institute of Research, 503 Robert Grant Avenue, Room # 2W88, Silver Spring, MD 20910, USA. Tel.: 301-319-9352; Fax: 301-319-9979; E-mail: tracy.rupp@amedd.army.mil

Melatonin is a hormone secreted by the pineal gland, the rhythm of which is controlled and generated by the intrinsic circadian pacemaker located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Pévet et al., 2006). The primary function of the melatonin profile in mammals is to convey information concerning daylength for organizing daylength-dependent seasonal functions. In diurnal mammals, melatonin secretion is associated with sleep and the low phase of the core temperature rhythm (Arendt, 2005, 2006). The administration of low (0.3–10 mg) doses of melatonin during the "biological day," when endogenous melatonin levels are low, can induce transient sleepiness or sleep and lower core body temperature (Arendt, 2005; Kräuchi et al., 2006); thus, melatonin has acute, sleep-promoting influences in humans.

Melatonin is detectable in plasma and saliva, and its metabolite, 6-sulfatoxymelatonin, is detectable in urine. The time of the evening onset of melatonin secretion under dim light conditions—dim light melatonin onset, or DLMO—has been used as a marker for the phase position of the circadian oscillator, usually from plasma samples obtained at frequent (about 30 min) intervals. Collecting saliva at similarly frequent intervals is a relatively noninvasive method of obtaining this measure compared to plasma.

In a recent review of research on alcohol and circadian rhythms, Danel and Touitou (2004) concluded that alcohol acts on the circadian timing system's biological clock, causing it to become "desynchronized." This conclusion was based on studies of daytime and nighttime melatonin levels in current or abstinent alcoholics (Danel & Touitou, 2004). Findings from such studies generally show suppressed melatonin levels in comparison to age-matched nonalcoholic controls (e.g., Kuhlwein et al., 2003). While the suppression of melatonin may not necessarily be indicative of changes in the circadian timing system, the authors' conclusions and the implications of the lowered melatonin level warrant investigation.

Results of the few studies examining the acute effect of alcohol on melatonin secretion have shown inconsistent results. One recent study did not show melatonin suppression with a 26 h alcohol session in healthy male volunteers without a history of alcoholism (Danel & Touitou, 2006). According to the authors, the absence of this finding may be related to the blood alcohol level (blood alcohol concentration was maintained between 0.5 and 0.7 g/l throughout), and the level at which ethanol might inhibit melatonin secretion is unclear (Danel & Touitou, 2006). Conversely, two studies of melatonin response to ethanol in young adult subjects without a history of alcoholism showed melatonin suppression in plasma or serum samples 6 to 10 h after acute alcohol administration, an effect not evident in the urinary metabolite (Ekman et al., 1993; Rodjmark et al., 1993). Melatonin suppression occurred with moderate to high doses of alcohol (Ekman dose = 0.5 g/kg or 1 g/kg body weight administered at 19 : 00 h; Rojdmark dose = 0.52 g/kg body weight administered at 18 : 00,

20 : 00, and 22 : 00 h), but not with a lower repeated dose of 0.34 g/kg body weight, administered at 18 : 00, 20 : 00, and 22 : 00 h (Rodjmark et al., 1993).

A number of methodological issues temper the interpretation of these findings:

1. Pre-study sleep-wake history was not controlled, leaving open the possibility that alcohol was administered at different circadian phases among participants and between dosing conditions.
2. Laboratory environmental control of dim light level was minimal before sleep.
3. Sample sizes were small.
4. Sex differences were not assessed.

The goal of this study was to investigate the acute effects of alcohol on salivary melatonin levels in a larger sample with stable prior sleep-wake pattern in a controlled study setting. The addition of these controls provided better opportunity to determine whether a moderate evening dose of alcohol suppresses melatonin levels and to identify the temporal association of alcohol to melatonin suppression. Sex differences were also examined.

METHODS

Participants

Participants were recruited through radio advertisements, flyers, posters, and appearances at community events. All procedures were approved by Lifespan Hospital Institutional Review Board for the Protection of Human Subjects and were consistent with the standards and ethical principles for research on biological rhythms on humans (Touitou et al., 2006). All subjects were paid for their participation. Nine men and 20 women, ages 21 to 25 ($M = 22.6$, $SD = 1.2$) yrs, completed the study. Participants were screened by telephone for the following exclusion criteria: chronic major medical illness; diagnosed sleep disorder; history of head trauma or brain injury; current illness; and current use of psychoactive agents or other drugs that could affect the sleep/wake cycle or daytime sleepiness/alertness. Participants were also excluded for travel beyond three time zones or shift work within three months of the study.

Procedures

After screening and study orientation, participants underwent a minimum of 10 nights on a stabilized sleep schedule before in-lab sessions

and remained on that schedule throughout the study, except as indicated below. The sleep stabilization schedule required that participants be in bed with lights out, attempting to sleep with eyeshades on, at the same time each night for a duration of either 8.5 or 9 h. Hours in bed, bedtimes, and rise times were determined based on participants' usual at-home sleep schedules and took into account other scheduling constraints (e.g., work or class schedules). Participants wore an actigraph (Mini Motionlogger BMA-32, Ambulatory Monitoring, Inc., Ardsley, New York, USA), called the laboratory's time-stamped answering machine every evening and morning, and completed a sleep diary throughout the study as a check for compliance to the sleep schedule. One participant was dropped for noncompliance with the schedule.

Each participant spent three nights in the lab, keeping the stabilized sleep schedule on the second and third nights in the lab and all intervening non-lab nights. Subjects' bed and rise times were delayed by 30 min on the first (adaptation) night. Lab nights included one adaptation night, followed by a placebo night and an alcohol night in counterbalanced order and separated from each other by 5–7 nights. To minimize expectation, participants were told they would receive an alcoholic drink, either of a low or moderate amount, on alcohol and placebo nights.

Participants arrived 6 h before stabilization bedtime and were given a meal and instrumented with electrodes for polysomnographic (PSG) monitoring. Thirteen saliva samples (2 ml) using plain cotton salivettes (Sarstedt Inc., Newton, North Carolina, USA) were obtained beginning 5 h before stabilized bed time. Six samples were taken before and seven after beverage administration at $\sim$30 min intervals. Light levels were $<$20 lux at all times during in-lab sessions. Participants sat for 5 min before each saliva sample. Saliva was extracted from the cotton by centrifugation, and samples were frozen (-20°F) and subsequently thawed for melatonin radioimmunoassay. Details of the radioimmunoassay are available elsewhere (Carskadon et al., 1999).

Alcohol (vodka: 0.54 g/kg for men and 0.49 g/kg for women) was mixed with chilled tonic water in a 1:4 ratio and consumed in three equal portions over 30 min, ending 1 h before stabilized bedtime. The dose was targeted for a Breath Alcohol Concentration (BrAC) of 0.05 g%, the equivalent of two to three standard drinks. For comparison, in certain European countries as well as regions in the United States, driving with a BrAC of 0.05 g% can result in a charge of driving while impaired, and a BrAC of 0.08% is considered driving while intoxicated. The same volume of tonic water as the total alcohol drink was given for the placebo condition. All drinks were prepared with a lime wedge and three drops of vodka floated on top.

Participants rinsed their mouths with alcohol-free mouthwash before consuming beverages. BrAC in g% was measured with an Alco-Sensor

IV Breathalyzer (Intoximeters, Inc., Richmond, Virginia, USA) when participants arrived and every 20 to 30 min after beverage administration until bedtime.

Analyses

Dim light melatonin onset (DLMO) phase was assessed for all participants by linear interpolation between rising values, crossing a threshold value of 4 pg/ml (Carskadon et al., 1999). One participant's DLMO was not calculated because his melatonin levels did not surpass threshold. We performed repeated measures analysis of variance on DLMO phase with a within-subjects factor condition and sex as a between-subjects factor. Melatonin levels following beverage administration were analyzed using repeated-measures ANOVA with within-subject factors sample number and condition and sex as a between-subjects factor. Greenhouse-Geisser adjustment was used for all analyses, and an α of .05 was used to determine statistical significance.

RESULTS

No significant differences were found for DLMO phase as a function of condition or sex, nor was the sex by condition interaction significant. Mean (SD) DLMO phase occurred 39.7 ($\pm$66) min before the end of beverage administration, which was 99.7 min before the stabilized bedtime in the placebo condition. For the alcohol condition, DLMO was 36 ($\pm$65) min before the end of beverage administration, 96 min before bedtime. Thus, beverage administration occurred at the same phase position for both conditions.

As shown in Figure 1, a significant main effect of condition manifested as the suppression of melatonin levels with alcohol ($F_{1,27}$; $p < .03$). Post-hoc analysis indicated suppression at samples 10 and 11 that occurred 140 and 190 min after alcohol administration. Melatonin levels were reduced by an average of 19% for sample 10 and 15% for sample 11. Mean (SD) BrACs at the time of these samples were 0.026 ($\pm$.008) and 0.016 ($\pm$.01) g%, respectively, corresponding to the elimination phase of alcohol metabolism. Neither the main effect for sex nor any other interaction was significant.

DISCUSSION

The sleep stabilization protocol was successful in controlling DLMO phase across conditions, which allows us to identify the effects of alcohol on salivary melatonin at a fixed dose, consistent circadian phase, and stable timing relative to sleep schedule. The time course of melatonin

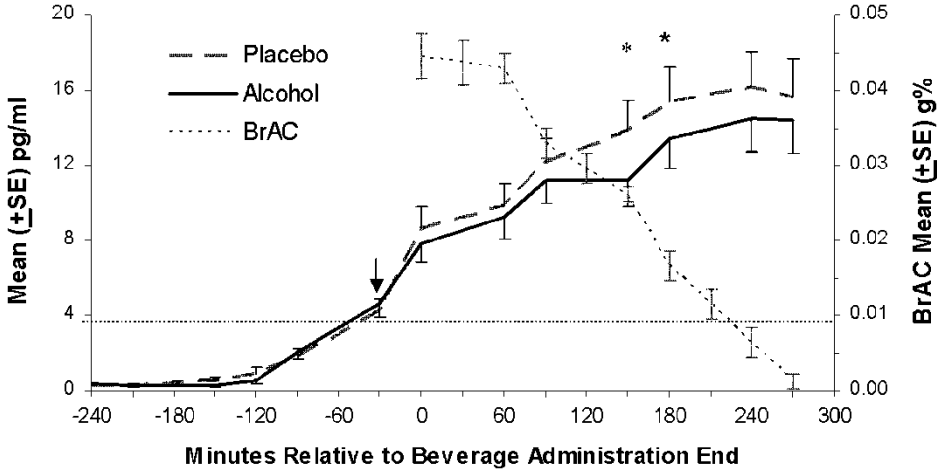

FIGURE 1 Mean and standard error of the mean melatonin values (pg/ml) are displayed for placebo (dashed line) and alcohol (solid line) conditions. The mean and standard error BrAC values on the alcohol night are depicted by the dotted line. Values for melatonin are labeled on the left y-axis and for BrAC on the right y-axis. Time on the x-axis is labeled in minutes relative to the end of beverage administration, which is time 0; the stabilized at home bedtime is +60 minutes. Asterisks indicate significant ($p < .05$) differences in melatonin levels between conditions. The arrow indicates DLMO phase.

suppression lagged alcohol administration by roughly 2.5 to 3 h and was a relatively fleeting effect occurring during the alcohol elimination phase in association with a rather low level of intoxication. In this regard, the melatonin suppressing effect of alcohol differs from the more immediate suppression caused by light. The mechanism of melatonin suppression by light involves the suppression of N-acetyltransferase, the enzyme that converts serotonin to melatonin. The interactions of alcohol and neurochemical systems are not yet fully understood; no known alcohol effect on brain neurotransmission explains its melatonin-suppressive effect.

The amount of suppression in the current study was comparable to the Rojdmark et al. (1993) observations, but lower than the 41% reduction reported by Ekman and colleagues (1993). In addition, the timing of suppression occurred earlier in the present study (i.e., 2.3–3.1 h versus 4–10 h in the previous studies). This latter difference may be due in part to differences in light conditions (i.e., continuous dim light in our study and dim light only at bedtime in the others). This difference is likely not due to dosage, as the effect was seen in the Ekman et al. (1993) study with the dose of 0.52 g/kg administered at 19:00 h, in addition to effects seen with the larger doses of 1 g/kg and 0.52 g/kg administered at 18:00, 20:00, and 22:00 h in the Rodjimark et al. (1993) study.

The present results showed that a moderate dose of alcohol was associated with a modest suppression of melatonin for a brief duration. The

limited suppression of melatonin at this dose is unlikely to have a strong effect on the circadian system; furthermore, an immediate negative impact of acute melatonin suppression on sleep was not evident in a subset of participants (Van Reen et al., 2006). On the other hand, the potential for interactions among alcohol, circadian timing, and sleep disruption may be enhanced with higher doses of alcohol taken late in the evenings, which is when many young adults typically time heavy alcohol consumption. With a pattern of heavy weekend night drinking, for example, melatonin suppression could contribute to a weekend circadian phase delay, thus perhaps exacerbating "Sunday night insomnia." Indeed, in susceptible persons, this pattern of drinking might also provoke a cycle of drinking to ameliorate sleep problems. Given that the current study used a moderate evening dose resembling social drinking, however, conclusions of the work should be generally limited to social/moderate drinking in a nonalcoholic population.

Phase shifting was not assessed in our study, and the suppression of melatonin does not necessarily mean that a phase shift will occur, although such an association is reasonable based on findings from previous animal work. For example, Rosenwasser et al. (2005) assessed the effects of chronic ethanol on the response of the circadian pacemaker to brief light pulses in rats and found that control animals displayed a marked period-shortening aftereffect in comparison to ethanol treated animals following a light pulse, thus suggesting that the chronobiological effects of ethanol are due in part to alterations at the level of the underlying circadian pacemaker.

We also note that melatonin suppression has been associated with breast cancer risk in female shiftworkers (Hansen, 2001; Schernhammer et al., 2001; Stevens & Davis; 1996). Stevens et al. (2000) hypothesized that melatonin inhibition may be associated with increased estrogen, increasing the risk of breast cancer. Given the increasing use of alcohol by young women (Mancinelli et al., 2006), a link from nighttime alcohol use to melatonin suppression may point to a heightened breast cancer risk in drinking women.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health (AA 13252) to Mary A. Carskadon, Ph.D. The authors would like to thank our consultants on the project, Dr. Tim Roehrs, Dr. J. Todd Arnedt, Dr. Robert Swift, and Dr. Peter Monti. Thanks also go to Denise Maceroni for saliva assays, and to Stephanie Crowley, M.S., and Jennifer Maxwell for assistance with data analysis and compilation. The assistance of Eliza Van Reen, M.S., the staff at the E.P. Bradley Sleep Lab, and Brown University students in data collection is gratefully acknowledged.

REFERENCES

Arendt J. (2005). Melatonin: characteristics, concerns, and prospects. *J. Biol. Rhythms* 20:291–303.
Arendt J. (2006). Melatonin and human rhythms. *Chronobiol. Int.* 23:21–37.
Carskadon MA, Labyak SE, Acebo CA, Seifer R. (1999). Intrinsic circadian period of adolescent humans measured in conditions of forced desynchrony. *Neurosci. Lett.* 260:129–132.
Danel T, Touitou Y. (2004). Chronobiology of alcohol: From chronokinetics to alcohol-related alterations of the circadian system. *Chronobiol. Int.* 21:923–935.
Danel T, Touitou Y. (2006). Alcohol consumption does not affect melatonin circadian synchronization in healthy men. *Alcohol Alcohol* 41:386–390.
Ekman A, Leppaluoto J, Huttunen P, Aranko K, Vakkuri O. (1993). Ethanol inhibits melatonin secretion in healthy volunteers in a dose-dependent randomized double blind cross-over study. *J. Clin. Endocrinol. Metab.* 77:780–783.
Hansen J. (2001). Increased breast cancer risk among women who work predominantly at night. *Epidemiology* 12:74–77.
Kräuchi K, Cajochen C, Pache M, Knoblauch V, Blatter K, Wirz-Justice A. (2006). Thermoregulatory effects of melatonin in relation to sleepiness. *Chronobiol. Int.* 23:475–484.
Kuhlwein E, Hauger RL, Irwin MR. (2003). Abnormal nocturnal melatonin secretion and disordered sleep in abstinent alcoholics. *Biol. Psychiatry* 54:1437–1443.
Mancinelli R, Binetti R, Ceccanti M. (2006). Woman, alcohol and environment: emerging risks for health. *Neurosci. Biobehav. Rev.* 31:246–253.
National Sleep Foundation. (2000). *Sleep in America poll*. Washington, DC.: National Sleep Foundation. See http://www.sleepfoundation.org.
Pévet P, Agez L, Bothorel B, Saboureau M, Gauer F, Laurent V, Masson-Pévet M. (2006). Melatonin in the multi-oscillatory mammalian circadian world. *Chronobiol. Int.* 23:39–51.
Rojdmark S, Wikner J, Adner N, Andersson D, Wetterberg L. (1993). Inhibition of melatonin secretion by ethanol in men. *Metab. Clin. Exp.* 42:1047–1051.
Rosenwasser AM, Logan RW, Fecteau ME. (2005). Chronic ethanol intake alters circadian period-responses to brief light pulses in rats. *Chronobiol. Int.* 22:227–236.
Schernhammer ES, Laden F, Speizer FE, Willet WC, Hunter DJ, Kawachi I, Colditz GA. (2001). Rotating night shifts and risk of breast cancer women participating in the nurses' health study. *J. Natl. Cancer Inst.* 93:1563–1568.
Stevens RG, Davis S. (1996). The melatonin hypothesis: electric power and breast cancer. *Environ. Health Perspect.* 104:135–140.
Stevens R, Davis S, Mirick D, Kheifets L, Kaune W. (2000). Alcohol consumption and urinary concentration of 6-sulfatoxymelatonin in healthy women. *Epidemiology* 11:660–665.
Touitou Y, Smolensky MH, Portaluppi F. (2006). Ethics, standards, and procedures of animal and human chronobiology research. *Chronobiol. Int.* 23:1083–1096.
Van Reen E, Arantes H, Rupp TL, Maxwell JL, Acebo C, Carskadon MA. (2006). Residual effects of a moderate dose of alcohol on sleep stages of young men. *Sleep* 29:A46–A47.