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Effects of Sleep and Sleep Deprivation on Catecholamine And Interleukin-2 Levels in Humans: Clinical Implications*

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ABSTRACT

The objective of this study was to evaluate the effects of nocturnal sleep, partial night sleep deprivation, and sleep stages on catecholamine and interleukin-2 (IL-2) levels in humans. Circulating levels of catecholamines and IL-2 were sampled every 30 min during 2 nights: undisturbed, baseline sleep and partial sleep deprivation-late night (PSD-L; awake from 0300–0600 h) in 17 healthy male volunteers. Sleep was monitored somnopolygraphically. Sleep onset was associated with a significant ($P < 0.05$) decline of circulating concentrations of norepinephrine and epinephrine, with a nocturnal nadir that occurred 1 h after nocturnal sleep. On the PSD-L night, levels of norepinephrine and epinephrine significantly ($P < 0.05$) increased in association with nocturnal awakening. During stage 3–4 sleep, levels of norepinephrine, but not epinephrine, were significantly lower ($P < 0.05$) compared to average levels during the awake period, stages 1–2 sleep, and rapid eye movement sleep. Nocturnal levels of circulating IL-2 did not change with sleep onset or in relation to PSD-L or the various sleep stages.

We conclude that sleep onset is associated with changes in levels of circulating catecholamines. Loss of sleep and disordered sleep with decreases in slow wave sleep may serve to elevate nocturnal catecholamine levels and contribute to cardiovascular disease. (*J Clin Endocrinol Metab* **84:** 1979–1985, 1999)

INCREASING evidence suggests that sleep-wake activity has a role in the homeostatic regulation of the sympathetic nervous system (SNS), with possible consequences for the onset of cardiovascular disorders. Rates of sudden cardiac death, myocardial infarction, and ischemic stroke are lowest during nocturnal sleep, then peak at the end of sleep or in the morning after awakening (1–3). Likewise, disordered sleep due to sleep apnea results in increases in the incidence of hypertension (4–6).

These clinical observations suggest that sleep may be linked to SNS activity. Indeed, Somers *et al.* continuously recorded sympathetic outflow to the muscle vascular bed with microneurographic methods during sleep and found decreases during nonrapid eye movement (non-REM) slow wave sleep and increases during nocturnal REM sleep compared to values during the awake period (7). However, the relationship between sleep and measures of norepinephrine and epinephrine, an index for SNS activity, remains poorly defined due to the limited number of studies and considerable heterogeneity in results (8, 9).

Because sleep has been infrequently monitored, it is not known whether the nocturnal drop of catecholamines is related to circadian- or sleep-dependent processes (10–14). Even fewer data are available concerning differences in catecholamines across sleep stages, and what data are available are discrepant in detecting norepinephrine and epinephrine differences during wakefulness, slow wave sleep, and REM sleep (12, 14, 15). Whereas both Somers *et al.* (7) and Vgontzas and colleagues (16) found a positive association between SNS activity and REM sleep, Dodt *et al.* colleagues recently reported no change in norepinephrine levels with morning awakening and decreases in catecholamines during REM sleep compared to levels during slow wave sleep (17). Moreover, sleep deprivation strategies that experimentally test the relationship between sleep and SNS activity have employed protocols that were prolonged in duration and relied on assessment of urinary catecholamines (10, 11, 18, 19) or a single plasma catecholamine determination after a night of total sleep loss (20).

Sleep is also believed to regulate the immune system (21). Born and colleagues have shown that stimulated *ex vivo* production of interleukin-2 (IL-2) is enhanced during sleep compared to that while awake and that this effect is dependent on sleep, rather than circadian processes (22, 23). Conversely, sleep loss that occurs in association with partial night sleep deprivation induces decreases in the stimulated production of IL-2 and natural killer cell responses (24, 25). However, almost no data are available regarding changes in *in vivo* immune variables, particularly in relation to sleep activity and specific sleep stages. In six humans, Moldofsky *et al.* reported that the onset of sleep and slow wave sleep were associated with increases in circulating concentrations of IL-1 followed by elevations of IL-2 (26). Other studies have

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found that patients with nocturnal sleep disturbance show altered circulating concentrations of tumor necros factor-α and IL-6 (27), and exogenous iv doses of IL-6 induce decreases in slow wave and REM sleep (28).

To address these questions regarding the effects of nocturnal sleep, sleep loss and sleep stages on the sympathetic and immune systems, we studied changes in circulating levels of catecholamines and IL-2 in human volunteers over 2 nights, baseline and partial night sleep deprivation. We hypothesized that sleep onset would be associated with a decline of catecholamines and an elevation of IL-2 levels. Furthermore, during the night of partial night sleep deprivation, nocturnal awakening would be associated with increases in catecholamines, whereas IL-2 levels would decrease compared to those in the same interval during the baseline night. Circulating concentrations of norepinephrine and epinephrine would also be related to sleep stages with decreases in catecholamines during slow wave sleep consistent with the findings of Somers *et al.* (7). Finally, previous data from our laboratory have shown that partial night sleep deprivation induces a decrement in natural killer (NK) cell activity. Because NK activity is inhibited by β-adrenergic receptor activation and stimulated by IL-2, the present study explored the correlation between changes in NK activity and changes in catecholamine and IL-2 levels after sleep loss.

Subjects and Methods

Subjects

Male volunteers (n = 17) were recruited by the University of California-San Diego Mental Health Clinical Research Center (MHCRC) using a standardized search strategy that involved community outreach educational efforts and placement of advertisements in local newspapers and university newsletters. The subject sample was comprised of men who were 38.8 ± 11.4 (mean ± SD) yr old, 76.4% white, and, on the average, college educated.

Informed consent was obtained under University of California-San Diego Protocol 96095. Subjects then underwent a psychiatric and medical evaluation by a MHCRC psychiatric fellow-physician. This evaluation included a structured clinical interview for the Diagnostic and Statistical Manual of Mental Disorders III-R (29), medical history, and review of systems, physical examination, and screening laboratory tests (chemistry panel, complete blood count, thyroid function tests, and human immunodeficiency virus test). Subjects had no lifetime history of a mental disorder by Diagnostic and Statistical Manual of Mental Disorders III-R criteria (30) with the exception of 2 men who fulfilled criteria for alcohol abuse (not dependence) who had been in remission for greater than 6 months. All subjects were in good medical health. None had chronic medical conditions, such as diabetes mellitus, cancer, cardiovascular disease, or hypertension. No subject reported recent (<10 days) viral infections that could affect the immune measures. Within the 2 weeks before study enrollment, none of the subjects was using medications such as psychotropic medications or drugs (prostaglandin inhibitors or β-blockers) that could affect sleep structure, measures of catecholamines, or immune function. Within the last 6 months, 3 of the subjects for whom catecholamine profiles were obtained reported use of benzodiazepine medications, and 10 subjects reported use of nonprescription antihistamine and/or analgesic medications. However, none of these subjects reported any prescription/nonprescription drug use other than occasional aspirin and multiple vitamins within the 2 weeks before the sleep protocol. Laboratory tests (chemistry panel, complete blood cell count, and thyroid tests) were all within normal limits. All subjects were negative for human immunodeficiency virus antibodies. In addition to the above psychiatric and medical evaluation, subjects underwent an interview to evaluate past and current substance use histories. None of the subjects was a current cigarette smoker, none fulfilled criteria within the last 6 months for alcohol abuse or dependence, and no subject reported other substance use (marijuana, psychostimulants, barbiturates, *etc.*) within the last 6 months.

For evaluation of each subject's sleep-wake activity, a diary was used for 2 weeks before study entry; these data revealed a sleep-wake schedule consistent with the study protocol. None of the subjects worked a night shift, and all slept between 2200–0700 h, getting, on the average, 7.5 ± 0.7 h of sleep/night.

Procedures

Subjects participated in a 3-night sleep protocol. On the first night in the sleep laboratory, subjects adapted to the conditions of the laboratory. During this adaptation night, recordings of oxygen desaturation and tibial myoclonus were obtained to exclude subjects with either sleep apnea or nocturnal myoclonus. On the second night (baseline night), sleep electroencephalogram (EEG) recordings and nocturnal blood samples were obtained during an uninterrupted period of nocturnal sleep. On the third night [partial sleep deprivation-late night (PSD-L night)], sleep EEG recordings and nocturnal blood samples were again obtained. After a period of uninterrupted sleep, subjects were awakened at 0300 h and kept awake until 0600 h.

On the 2 experimental nights, subjects arrived at the laboratory at about 2000 h. Subjects then readied for sleep and had electrodes placed for EEG, electrooculography, and submental electromyography recordings. At 2100 h, an iv catheter was inserted into a forearm vein, and subjects rested in a supine horizontal position in an individual bedroom at the MHCRC. Subjects remained awake during this hour, and the first blood sample was obtained at 2200 h, 1 h after placement of the iv and maintenance of a supine position. We have previously demonstrated that circulating levels of catecholamines reach a stable resting baseline within 30 min after iv placement and rest (31). Lights were turned off between 2230–2300 h, and subjects were, on the average, asleep at 2300 h on the baseline night and at 2315 h on the PSD-L night. On the baseline night, subjects were awakened at 0600 h after the last blood draw if they were not already awake. On the PSD-L night, subjects were awakened immediately after the 0300 h blood draw and were kept awake by a sleep technician who monitored subject behavior and EEG. All subjects included in the present analyses remained supine throughout the entire nocturnal period on the baseline and PSD-L nights; a bedside urinal was used if subjects needed to urinate during the night.

For blood sampling, the iv catheter was connected to a long thin plastic tube that enabled blood collection from an adjacent room without disturbing the subject's sleep. Between blood samplings, continuous heparinized isotonic saline was infused, totaling about 1000 mL during each of the nocturnal periods. Blood was sampled every 30 min starting at 2200 h until 0600 h, and the removed blood volume totaled no more than 150 mL for each experimental night. Immediately after the blood was obtained, it was put into heparinized tubes, placed on ice, and centrifuged at 4 C. Plasma samples were then stored at −80 C until assay; IL-2 was assayed within 6 months, and catecholamine assays were completed within 1 yr of sample acquisition. After completion of serial nocturnal sampling, an additional aliquot of blood was obtained and placed in a heparinized Vacutainer tube (Becton Dickinson and Co., Rutherford, NJ) for assay of NK activity.

Sleep EEG measures were obtained during continuous recordings between 2200–0600 h during the adaptation, baseline, and PSD-L nights. Sleep data from the first night were not used in the analyses. Sleep records were visually scored according to the criteria of Rechtschaffen and Kales (32). Data from each 30-s epoch were entered into a computer program that tallies the summary statistics for each subject. Sleep onset was defined as the first minute of stage 2 or REM sleep, followed by at least 8 min of sleep in the next 9 min. A REM period was defined by not less than 3 consecutive min of REM sleep. Sleep efficiency was the ratio of total sleep time to the time in bed multiplied by 100. Sleep architecture was defined as the duration of time spent asleep in non-REM sleep, stages 1–4. REM density was an estimate of the number of eye movements per min during REM sleep, scored on a scale of 0–4/30-s epoch, but expressed on a scale of 0–8/min. Sleep research technicians were regularly tested for scoring reliability, and high standards were maintained: sleep latency (r = 0.96), REM latency (r = 0.99), REM density (r = 0.91), amounts of stages 3 and 4 (r = 0.85), and total sleep time (r = 0.99).

Assays

Catecholamines. Blood samples across the entire baseline and PSD-L nights were available for assay of norepinephrine and epinephrine in 12 subjects. Other subjects were missing plasma samples at 1 or more times across 1 of the nights due to technical problems in the collection of the sample.

Catecholamines were assayed by modification of the catechol-*O*-methyltransferase-based radioenzymatic assay (33). This technique uses a preassay concentration step that eliminates interfering substances and improves sensitivity 10-fold. The intraassay coefficients of variation for norepinephrine and epinephrine are 4% and 13%, respectively. The interassay coefficients of variation are 10% and 16%.

IL-2. Blood samples across the entire baseline and PSD-L nights were available for assay of serum concentrations of IL-2 in 17 subjects. Serum levels of IL-2 were quantitated using the IL-2-sensitive murine CTLL-2 line. The CTLL bioassay was employed rather than enzyme-linked immunosorbent assay due to nonspecific inhibitory effects of the serum on the enzyme-linked immunosorbent assay results.

The CTLL-2 cell line was obtained from American Type Culture Collection (Manassas, VA) and was maintained in culture with RPMI 1640 with 10% FCS (Sterile System, Logan, UT), 10 pg/mL IL-2 (natural human; Boehringer Mannheim, Indianapolis, IN), and 5×10^{-5} mol/L mercaptoethanol. The CTLL-2 cells were used on day 5 after initiating culture; at this time IL-2 was depleted, and most cells were in the resting phase. In addition, to ensure that the CTLL-2 were sensitive to IL-2, CTLL-2 cell cultures were recloned every 6–8 weeks.

The amount of IL-2 in the experimental sample was determined by measuring the proliferative response of 3×10^4 CTLL-2 cells after incubation at 37 C in 5% CO_2 for 18 h. Cells were then pulsed with [^{3}H]thymidine (New England Nuclear Corp., Boston MA) for 4 h, harvested, and counted in a direct β-counter (Packard Matrix, Meriden CT). Each bioassay established a standard response curve using varying known amounts of IL-2. Only those bioassays were analyzed in which a linear dose-response range was demonstrated. Furthermore, serum concentrations of IL-2 are low and are typically at the limits of bioassay sensitivity. Because the CTLL-2 standard curve was flat and nonlinear at low IL-2 doses, standard amounts of IL-2 were added to the experimental serum samples to yield a concentration of IL-2 that was in the measurable range and along the linear part of the standard dose-response curve. The amount of IL-2 in the serum was then determined by calculating the difference in the CTLL-2 response in the serum sample compared to that in the respective standard. To evaluate whether the bioassay yielded reproducible and reliable levels of IL-2 in the serum samples without possible effects of nonspecific serum inhibitors, the serum samples were diluted across a range of concentrations (1:4, 1:8, and 1:16) and yielded similar final IL-2 concentrations. The specificity of the IL-2 bioassay for IL-2 was verified using a polyclonal IL-2 antibody to natural human IL-2 (Boehringer Mannheim); titration of anti-IL-2 completely abrogated the proliferative CTLL-2 response. The methods for assay of NK activity have been previously reported (24).

Statistical analysis

Circulating levels of norepinephrine, epinephrine, and IL-2 during the baseline and PSD-L nights were profiled in reference to the individual time of sleep onset. To test for differences between the 2 nights (baseline and PSD-L) across the nocturnal period, a mixed design, repeated measures ANOVA was conducted. To evaluate the specific hypotheses regarding changes in circulating catecholamine and IL-2 levels in relation to sleep onset, sleep deprivation, and sleep stages, planned comparisons using paired *t* tests were conducted. Planned comparisons are considered the most appropriate test for evaluating specific hypotheses and allow for the extraction of information critical to the status of the research question (34). Thus, to test whether a decrease in circulating catecholamines occurred after sleep onset, paired *t* tests compared average awake and nocturnal nadir catecholamine levels. To evaluate whether an increase in IL-2 occurred after sleep onset, paired *t* tests compared average awake and nocturnal peak IL-2 levels. To evaluate whether awakening during the late part of the night from 0300–0600 h resulted in increases in catecholamines and decreases in IL-2, average levels of norepinephrine, epinephrine, and IL-2 during this time interval were compared between the baseline and PSD-L nights. To examine the effects of sleep stages, levels of norepinephrine, epinephrine, and IL-2 from each stage (awake, stages 1–2, stages 3–4, and REM) were obtained. *A priori* planned comparisons were used to test whether catecholamine and IL-2 levels differed between values obtained during stage 3–4 compared to the awake period and other sleep stages. Finally, to evaluate the association between changes in NK activity and circulating concentrations of catecholamines and IL-2 after PSD-L, the difference in NK activity between the baseline and PSD nights was calculated and then correlated with average late night circulating concentrations of norepinephrine, epinephrine, and IL-2 using nonparametric Spearman correlations.

Results

Table 1 shows the means for the various EEG sleep measures obtained during the baseline and PSD nights. These findings have been previously reported in our evaluation of the effects of partial sleep deprivation on NK activity (24). The subjects slept an average of 6.7 h until finally awakening on the baseline night, and this amount of total sleep was reduced to about 3.8 h on the PSD-L night. There was no difference in sleep efficiency, sleep latency, or relative amounts of the five sleep stages (stages 1–4 and REM sleep) between baseline and sleep deprivation nights. Measures of REM latency and REM density were also similar between the 2 nights. Together, the EEG measures indicate that the physiological structure of sleep was preserved during the PSD-L night, with only a loss of total sleep time.

Sleep onset and catecholamine and IL-2 levels

Across individuals who were time locked to sleep onset, circulating concentrations of norepinephrine showed a significant time effect ($F = 2.6$; $P < 0.01$), no night effect ($F = 0.10$; $P = 0.75$), and a time × night interaction ($F = 1.7$; $P <$

TABLE 1. EEG sleep measures during baseline and partial sleep deprivation-late night

	Baseline night mean (SD)	Partial sleep deprivation-night mean (SD)
Sleep Continuity		
Total sleep time (min)	400.2 (42.4)	225.5 (25.7)
Sleep efficiency (%)	87.7 (7.5)	86.8 (8.8)
Sleep latency (min)	11.0 (10.9)	13.3 (9.7)
Sleep Architecture		
Stage 1 sleep		
(min)	25.9 (14.9)	13.2 (9.9)
(%)	6.5 (3.6)	5.9 (4.3)
Stage 2 sleep		
(min)	250.8 (36.2)	147.3 (24.2)
(%)	62.9 (7.9)	65.6 (9.9)
Stage 3 sleep		
(min)	25.9 (20.2)	18.7 (16.8)
(%)	6.4 (4.9)	8.3 (7.4)
Stage 4 sleep		
(min)	13.5 (18.8)	9.4 (13.9)
(%)	3.8 (4.4)	4.1 (5.9)
REM measures		
REM sleep		
(min)	84.0 (22.1)	36.9 (14.5)
(%)	21.0 (10.9)	16.4 (8.8)
REM latency		
(min)	77.9 (40.4)	62.5 (20.9)
REM density		
(1st period)	1.7 (0.6)	1.7 (0.6)

0.05). The time effect indicated that circulating levels of norepinephrine changed significantly across the nocturnal period. During the baseline night, averaged norepinephrine plasma concentrations reached a nocturnal nadir (mean ± SD, 144.6 ± 56.2 pg/mL) 1 h after subjects fell asleep that was lower than the norepinephrine level obtained during the awake period before sleep onset when the subjects were also confined to a horizontal position (185.0 ± 68.1 pg/mL; t = 2.1; $P < 0.05$; Fig. 1A). Similar results were obtained for the PSD-L night (norepinephrine: nocturnal nadir, 156.8 ± 60.5 pg/mL; awake level, 204.5 ± 62.2 pg/mL; t = 2.7; $P < 0.05$).

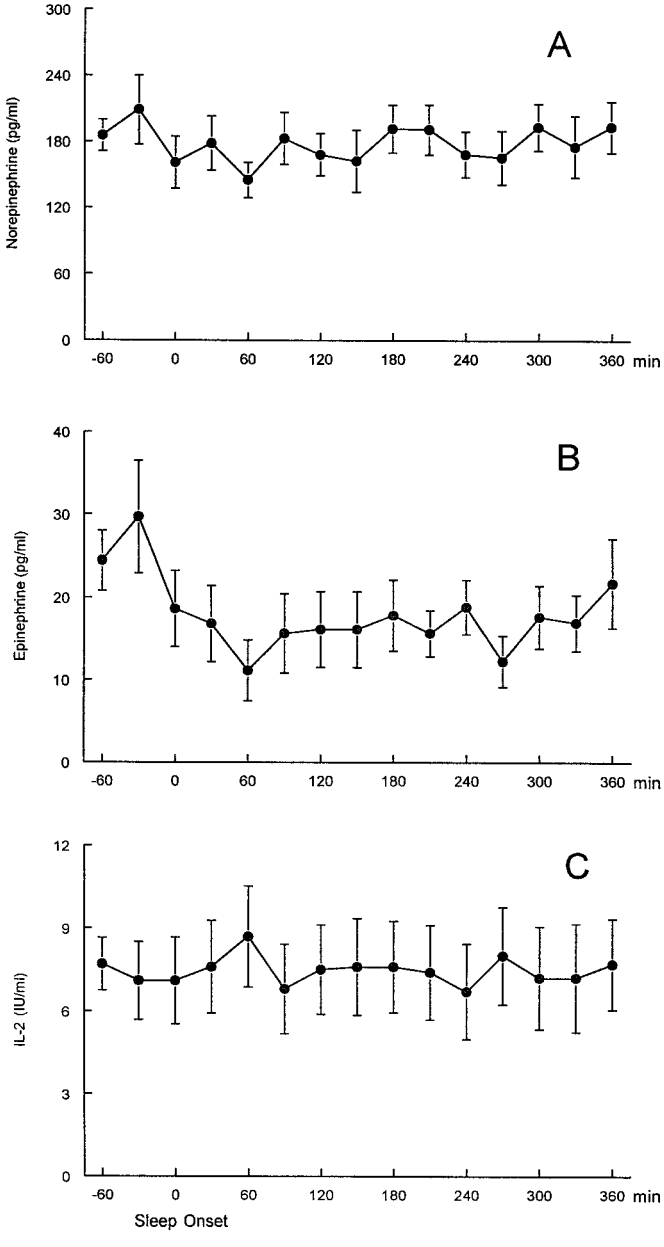

FIG. 1. Mean (±SEM) circulating levels of norepinephrine (A), epinephrine (B), and IL-2 (C) in subjects during the baseline night. Profiles were averaged across individuals time locked to sleep onset, indicated by the *vertical arrow*. Blood was drawn every 30 min. Subjects remained strictly in a horizontal position 60 min before the first blood sample.

Epinephrine levels were low, but well above 6 pg/mL, the lower limit of assay sensitivity. For circulating concentrations of epinephrine across subjects referenced to sleep onset, there was also a significant time effect (F = 2.2; $P = 0 < 0.01$) consistent with a decline of epinephrine across the night. No night effect (F = 0.18; P = 0.68) or time × night interaction (F = 1.4; P = 0.14) was found. Similar to the changes in norepinephrine, epinephrine concentrations during the baseline night reached a nocturnal nadir (11.0 ± 12.7 pg/mL) 1 h after sleep onset that was significantly lower than the awake level (24.2 ± 16.0 pg/mL; t = 5.4; $P < 0.001$; Fig. 1B). The PSD-L night showed an identical pattern of decline (epinephrine: nocturnal nadir, 13.7 ± 9.6 pg/mL; awake level, 21.0 ± 7.9 pg/mL; t = 2.3; $P < 0.05$).

Circulating levels of IL-2 across subjects referenced to sleep onset also showed a time effect (F = 2.1; $P < 0.01$), but no night effect (F = 0.07; P = 0.75) and no time × night interaction (F = 1.5; P = 0.09). To determine whether the time effect was due to an increase in IL-2 after sleep onset, the peak level of IL-2 level 1 h after sleep onset (8.7 ± 6.4 pg/mL) was compared to the awake level (7.3 ± 5.0 pg/mL). IL-2 levels were similar between the awake period and the nocturnal peak (t = 1.7; P = 0.06; Fig. 1C). Likewise, IL-2 levels were similar between awake and the nocturnal peak during the PSD-L night (IL-2: nocturnal peak, 7.9 ± 6.1 pg/mL; awake level, 7.1 ± 5.4 pg/mL; t = 1.1; P = 0.15). Additional *post-hoc* comparisons further evaluated the time effect for IL-2 and demonstrated that the peak level of IL-2 1 h after sleep onset was higher than the levels at 90 min (t = 3.3; $P < 0.01$) and 240 min (t = 2.1; $P < 0.05$) after sleep onset.

Sleep deprivation and catecholamine and IL-2 levels

For norepinephrine, the time × night interaction reported above indicated that there was a significant difference in the pattern of norepinephrine levels between the baseline and PSD-L nights. This interaction was due to an increase in the levels of norepinephrine that occurred in association with awakening during the late part of the PSD-L night. The average level of norepinephrine between 0300–0600 h was significantly higher on the PSD-L night (223.6 ± 77.2 pg/mL) compared to that obtained during the same time period on the baseline night (178.6 ± 62.3 pg/mL; t = 2.3; $P < 0.05$). There was no difference in the average level of norepinephrine obtained in the early part (2200–0300 h) of the baseline night (176.8 ± 59.6 pg/mL) compared to that on the PSD-L night (176.4 ± 62.6 pg/mL; t = 0.02; P = 0.98; Fig. 2A).

For epinephrine, the average level of epinephrine between 0300–0600 h also tended to be higher during the PSD-L night (25.2 ± 13.3 pg/mL) compared to the baseline night (18.1 ± 9.3 pg/mL), although this difference did not reach statistical significance (t = 1.9; P = 0.09; Fig. 2B). Average levels of epinephrine obtained in the early part of the night (2200–0300 h) were similar between the baseline (17.8 ± 13.6 pg/mL) and PSD-L nights (18.9 ± 9.3 pg/mL; t = 0.3; P = 0.74).

For IL-2, pairwise comparisons showed that IL-2 levels obtained from 0300–0600 h were similar for baseline (7.7 ± 6.8 pg/mL) and PSD-L nights (6.5 ± 4.8 pg/mL; t = 1.1; P = 0.29; Fig. 2C). Likewise, there was no difference in average levels of IL-2 during the early part (2200–0300 h) of the

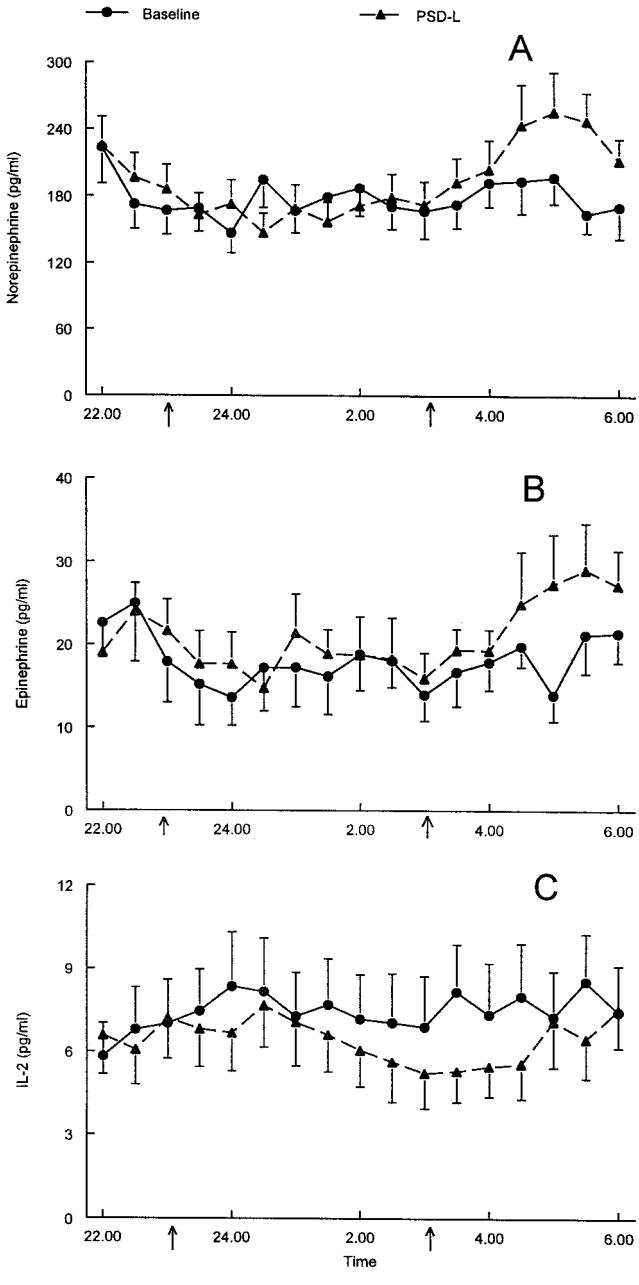

FIG. 2. Mean (±SEM) circulating levels of norepinephrine (A), epinephrine (B), and IL-2 (C) in subjects during the baseline and PSD-L nights. The *arrow* at 2300 h indicates the average time that the subjects were asleep on the baseline night; the *arrow* at 0300 h indicates the time that the subjects were awakened on the PSD-L night.

baseline (7.2 ± 6.4 pg/mL) and PSD-L nights (6.9 ± 5.2 pg/mL; $t = 0.33$; $P = 0.75$).

Sleep stages and catecholamine and IL-2 levels

The propensity of slow wave sleep to occur in the early part of the night together with nocturnal nadir of catecholamines 1 h after sleep onset suggested that there might be sleep stage-specific changes in SNS activity. Thus, levels of norepinephrine and epinephrine were compared between

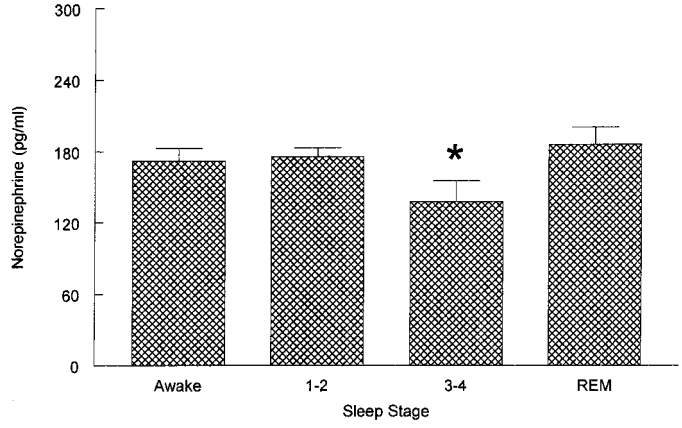

FIG. 3. Mean (±SEM) plasma levels of norepinephrine across the various sleep stages. *, $P < 0.05$, stages 3–4 sleep *vs.* awake period, stages 1–2 sleep, and REM sleep.

the awake period and various sleep stages over the nocturnal period. *A priori* comparisons demonstrated that circulating concentrations of norepinephrine were significantly lower ($F = 3.6$; $P < 0.05$) during stages 3–4 sleep compared to average levels during the awake period, stages 1–2 sleep, and REM sleep (Fig. 3). There was no difference in levels of norepinephrine found in any of the other sleep stages. Levels of epinephrine were similar during the awake period, stages 1–2 sleep, stages 3–4 sleep, and REM sleep ($F = 0.29$; $P = 0.59$). Furthermore, no difference in levels of IL-2 between the awake period and each of the sleep stages was found ($F = 0.23$; $P = 0.63$).

Relationship between sympathetic and immune changes during PSD-L

A previous report by our laboratory demonstrated that PSD-L is associated with a decrement in NK cell activity (24). Compared to levels obtained in the morning after a night of baseline sleep, mean NK lytic activity is reduced 45% after a night of PSD-L (24). The mechanisms that mediate the association between PSD-L and reduced NK activity are not known, although substantial evidence has shown that the release of catecholamines and β-adrenergic receptor activation results in a reduction of *ex vivo* levels of NK activity in humans (35). Alternately, the release of IL-2 serves as a potent inducer of cytotoxic activity (36). In the present study, the relationship between the change in NK activity and average circulating levels of catecholamines and IL-2 was explored using correlational analyses. Late night circulating concentrations of norepinephrine during PSD-L were negatively correlated with a change in NK activity from baseline to the PSD-L night ($\rho = 0.48$; $P = 0.05$). There was no association between late night levels of epinephrine or IL-2 and a change in NK activity, and early night levels of catecholamines or IL-2 were not correlated with a change in NK activity.

Discussion

Sleep onset is associated with a decline in plasma levels of norepinephrine and epinephrine, with a nocturnal nadir occurring 1 h after sleep onset. In contrast, awakening during

partial night sleep deprivation resulted in increases in norepinephrine and epinephrine. Finally, circulating norepinephrine levels were lower during slow wave non-REM sleep than during the awake period, stages 1–2 sleep, or REM sleep.

These data implicate sleep in the nocturnal regulation of sympathetic activity. First, the decrease in circulating catecholamines during the nocturnal period occurred after sleep onset and cannot be explained by postural changes, as all subjects maintained a supine horizontal position that began 1 h before blood sampling and was continued throughout the night. Dodt *et al.* also found that falling asleep produced a decrease in epinephrine (17), and the present study extends these observations to norepinephrine levels. Second, experimental induction of sleep loss resulted in an increase in norepinephrine. Sleep, rather than a circadian pacemaker, influences nocturnal declines in norepinephrine. Finally, the nocturnal nadir for norepinephrine occurred during the first part of the night, in which there is a propensity for slow wave sleep, and norepinephrine levels were lowest during slow wave sleep compared to those during the awake period and other sleep stages. The present observations linking decreases in norepinephrine and slow wave sleep are consistent with those of Somers *et al.*, who recorded single sympathetic nerve fiber activity in skeletal muscle during sleep (7). Sympathetic burst activity and amplitude decreased during sleep, with lowest levels during slow wave sleep. Hemodynamic measures also revealed decreases during non-REM sleep, but no change in heart rate or blood pressure during REM sleep (7).

There is considerable heterogeneity in the studies linking sleep stages and catecholamines. Some studies have failed to find differences across sleep stages (11), whereas others have reported decreases in norepinephrine during REM sleep (17, 37), but not during slow wave sleep (17). These discrepant results may be explained in part by differences in sampling rates, as suggested by Dodt and colleagues (17). Longer sampling intervals of 20–30 min may not provide as reliable an estimate of plasma catecholamines as shorter sampling periods and may miss changes in catecholamines that occur during sleep periods that are short in duration. However, in the present study with a sampling interval of 30 min, a decrease in plasma norepinephrine was found during slow wave sleep even though these periods were infrequent and short compared to REM sleep in this group of middle-aged subjects. Differences in assay sensitivity may also explain the heterogeneity in results linking norepinephrine to sleep stages; the catecholamine assay used in the present study is more sensitive than those used in prior studies.

Ex vivo measurement of IL-2 production by lymphocytes has revealed increases during sleep and decreases during and after sleep loss (22, 25). The present study extended these data by assay of circulating *in vivo* concentrations of IL-2. Although IL-2 levels varied during the sleep period, there was no association between sleep activity and changes in circulating levels of IL-2. Sleep onset was not followed by increases in circulating IL-2, nor was there any significant change in serum concentrations of IL-2 in relation to sleep deprivation or sleep stages. The lack of a consistent influence of sleep on circulating concentrations of IL-2 (21, 26, 38), as opposed to IL-2 production, is most parsimoniously explained by the local release of IL-2 at cellular sites where it is rapidly used, degraded, or bound to soluble IL-2 receptors for uptake. Thus, *in vivo* circulating concentrations of IL-2 may not reliably demonstrate the impact of sleep on the expression and release of this cytokine.

Considerable evidence has shown that sleep deprivation is associated with alterations of *ex vivo* immune parameters, such as NK activity (24, 39). Among the mechanisms possibly mediating the effects of sleep on changes in NK activity, the release of sympathetic catecholamines has been suggested (35). In the present study, we found that nocturnal elevations of norepinephrine during partial night sleep deprivation correlated with the decline of NK activity from baseline to PSD-L nights, consistent with observations that circulating levels of norepinephrine and β-adrenergic receptor activation correlate with a reduction of NK activity (35). Given the effect of sleep on endocrine factors, the roles of other mediators in the sleep immune relation should also be explored. For example, GH is related to slow wave sleep, and this neuroendocrine hormone has been found to alter multiple aspects of cellular immunity (40, 41).

Sleep maintenance problems, loss of slow wave sleep, and waking up feeling exhausted have been implicated as risk factors associated with a first myocardial infarction (42, 43, 44). Whether elevations in nocturnal catecholamines that occur in association with sleep loss or possibly with loss of slow wave sleep contribute to the onset of cardiovascular events is not yet known. However, sympathoadrenal activation produces a combination of transient hemodynamic, vasoconstrictive, and prothrombotic processes that are thought to increase the risk of plaque disruption and thrombosis, the final pathway of most myocardial infarctions (45, 46).

In conclusion, sleep loss is endemic in society (47), and loss of sleep during only part of the night is one of the most common complaints of persons who experience environmental or psychological stress (48, 49), travel across time meridians, engage in shift work (48), or suffer from a psychiatric disorder (50, 51). The present study indicates that awakening during the night with only a modest amount of sleep loss enhances sympathoadrenal activity. This provides a mechanism that could potentially link sleep loss with cardiovascular disorders.

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