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Nocturnal Plasma Levels of Cytokines in Healthy Men

Susanne Gudewill, Thomas Pollmächer, Helmut Vedder, Wolfgang Schreiber, Klaus Fassbender, and Florian Holsboer

Max Planck Institute of Psychiatry, Clinical Institute, Department of Psychiatry, Kraepelinstrasse 10, W-8000 München 40, Federal Republic of Germany

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Summary. Nocturnal cytokine levels were measured serially in 12 healthy male volunteers for 12 h, including 8 h of polygraphically monitored nocturnal sleep. Plasma concentrations of interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were determined in 30-min intervals by enzyme-linked immunoadsorbant assays. In some subjects cytokines were not detectable at all. In the remaining volunteers (27% for IL-1β, 58% for IL-6 and TNF-α, respectively) occasional values near to the detection limits (DL) of the assays could be measured. With respect to IL-1β and IL-6, plasma levels above the DL were significantly more frequent during sleep than during the preceding time of wakefulness. No temporal association with NREM or REM episodes could be shown. TNF-α values above the DL were randomly distributed across the 12-h period investigated. It is concluded that in a considerable percentage of healthy subjects small amounts of cytokines are released at night. Release of IL-1β and IL-6 is temporally associated with sleep, whereas the release of TNF-α is not. It remains to be established whether nocturnal cytokine release reflects either an interaction between sleep and host defense mechanisms or a sleep-independent circadian rhythmicity.

Key words: Cytokines – Interleukin-1β – Interleukin-6 – Tumor-necrosis-factor – Sleep

Introduction

There is ample evidence that acute systemic infection and bacterial cell wall components promote sleep in animals (Krueger et al. 1986; Toth and Krueger 1988; Krueger and Johannsen 1989). Activation of host defense mechanisms influences sleep probably via cytokines like interleukin-1 (IL-1) and tumor necrosis factor (TNF), which by themselves are somnogenic (Krueger et al. 1984; Tobler et al. 1984; Shoham et al. 1987).

Correspondence to: Thomas Pollmächer

Moreover, cytokines have been supposed to be involved in the physiological regulation of sleep and wakefulness (Krueger 1990). This view is supported by studies indicating that IL-1β levels are enhanced during sleep in the cerebrospinal fluid of cats (Lue et al. 1988) and that peaks in IL-1- and IL-2-like activity occur shortly after sleep onset in healthy humans (Moldofsky et al. 1986). Other cytokines, however, have not been studied until now with respect to a possible association of their release with sleep.

We measured the plasma levels of the somnogenic cytokines IL-1β and TNF-α together with those of interleukin-6, which is pyrogenic, but not somnogenic in animals (Opp et al. 1989), by highly sensitive and specific enzyme-linked immunoadsorbant assays in 12 healthy male volunteers during a 12 h period including 4 h of wakefulness followed by 8 h of nocturnal sleep.

Materials and Methods

Twelve male volunteers (mean age 26.1 years, range 21–30 years) gave written informed consent to take part in the investigation. The experimental protocol had been approved by the Ethics Committee for Human Experimentation at the Max Planck Institute of Psychiatry. Prior to the enrollment in the study, subjects had been screened carefully by detailed medical history and sleep habits evaluation, physical examination, laboratory investigation, ECG and EEG to be free of acute and chronic illness.

Each experimental session was preceded by a night of adaptation to sleep laboratory conditions (2300 to 0700 hours). In the following morning complete blood count, white cell differential count and physical examination were repeated to exclude acute infection. Subjects were requested to lie down in bed at 1730 hours. An intravenous cannula was inserted into an antecubital forearm vein and connected to an infusion pump in the adjacent room as described in detail elsewhere (Holsboer et al. 1984). Serial venous blood samples were taken in 30-min intervals from 1900 till 0700 hours. Blood was stabilized with Na-EDTA (1 mg/ml) and aprotinine (300 K.I.E./ml blood) and centrifuged immediately for 10 min (2600 g at 4°C). Aliquoted plasma samples were frozen and stored at −20°C until they were assayed for IL-1β, IL-6 and TNF-α. Polygraphic monitoring of sleep and wakefulness according to Rechtschaffen and Kales (1968) was started at 1900 hours. Subjects were requested to stay awake till lights were switched off at 2300 hours, when they were instructed to sleep as long as they

Table 1. Sleep parameters (mean ± SD)

Time in bed (min)	511 ± 48
Sleep period time (min)	477 ± 57
Total sleep time (min)	435 ± 39
Sleep efficiency index (%)	90 ± 7
Number of awakenings	16 ± 9
Sleep onset latency (min)	26 ± 19
REM latency (min)	52 ± 32
SWS latency (min)	26 ± 27
% of Time in bed	
Stage 1	8.4 ± 3.1
Stage 2	42.2 ± 6.7
Stage 3	6.8 ± 1.5
Stage 4	3.9 ± 4.4
REM	22.5 ± 4.0
Awake	18.0 ± 10.1
Movement time	1.2 ± 0.5

wanted to. Scoring of polygraphic sleep recordings was done visually according to established criteria (Rechtschaffen and Kales 1968). As shown in Table 1, sleep parameters were within the usual range for healthy volunteers, indicating that night sleep was not grossly disturbed by the blood sampling procedure.

Plasma levels of IL-1β, IL-6 and TNF-α were all determined by enzyme-linked immunoadsorbant assays (Medgenix Diagnostics, Brussels, Belgium). According to the supplier, the minimum detectable concentration for IL-6 and TNF-α is 3 pg/ml, for IL-1β it is 2 pg/ml; in addition, cross-reactivity among the cytokines investigated has been excluded. Samples of each individual were measured in the same kit. All assays were done in duplicate. For technical reasons IL-1β could not be assayed in one subject. The intra-assay coefficient of variation (CV) for IL-1β was 3.4% (for the low) and 2.2% (for the high controls) and the inter-assay CV was 4.6% and 3.1%, respectively. The intra-assay CV for IL-6 was 5.6% (4.7%) and the inter-assay CV 10.2% (8.5%). For TNF-α the intra-assay CV was 5.1% (3.7%) and the inter-assay CV 5.2% (2.0%), respectively.

Results

In some subjects, cytokines were not detectable at all during the whole 12-h period investigated. In the remaining volunteers (3/11[27%] for IL-1β, 7/12[58%] for IL-6 and 7/12[58%] for TNF-α) values above the detection limits (DL) of the assays ranged from 2.2 to 4.6 pg/ml for IL-1β, from 3.3 to 19.1 pg/ml for IL-6 and from 3.0 to 16.7 pg/ml for TNF-α. Because all of these values were close to the DL, we decided to dichotomize them for further data analysis into those below and those above the DL. As shown in Table 2, only in 2 subjects all three cytokines could be detected, whereas in 2 subjects IL-1β and IL-6, in 5 subjects IL-6 and TNF-α, and in 2 subjects IL-1β and TNF-α were found.

As illustrated by Fig. 1 and Table 2, IL-1β and IL-6 values above the DL occurred almost exclusively during nocturnal sleep, whereas TNF-α values above the DL were equally distributed across the experimental time period. This was confirmed by a binomial test assuming that, if values above the DL would occur randomly across the 12 h, their expected relative frequency would be 36% during the 4 h until 2300 hours (9 of 25 time points) and 64% during the 8 h thereafter (16 of 25 time points). The binomial test yielded a significant deviation from the expected frequencies for both IL-1β and IL-6 ($P < 0.01$), but not for TNF-α. In addition, the percentage of values above the DL was higher after 2300 hours than before with respect to IL-6 (17, SD 17% vs. 2, SD 4%, $N = 7$, $P < 0.05$), but not with respect to TNF-α (33, SD 32% vs. 29, SD 36%, $N = 7$, n.s.) as assessed by the Wilcoxon matched-pairs signed-ranks test. The figures for IL-1β were 27, SD 31% vs. 0, SD 0%; statistical analysis, however, was not performed, because IL-1β values above the DL were found in only 3 subjects.

To assess whether IL-1β or IL-6 release is temporally related to the NREM/REM cycle, we determined for

Table 2. Number and percentage of cytokine values above the detection limit before and after 2300 hours

	IL-1β				IL-6				TNF-α			
	1900–2300		2330–0700		1900–2300		2330–0700		1900–2300		2330–0700 hours	
Subject	*N* (%)											
1	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
2	0	(0)	0	(0)	0	(0)	0	(0)	4	(44)	9	(56)
3	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)
4	0	(0)	0	(0)	0	(0)	1	(6)	2	(22)	5	(31)
5	0	(0)	0	(0)	1	(11)	8	(50)	0	(0)	0	(0)
6	0	(0)	2	(13)	0	(0)	2	(13)	9	(100)	15	(94)
7	0	(0)	1	(6)	0	(0)	1	(6)	0	(0)	1	(6)
8	0	(0)	0	(0)	0	(0)	1	(6)	0	(0)	0	(0)
9	0	(0)	0	(0)	0	(0)	5	(31)	3	(33)	3	(19)
10	0	(0)	0	(0)	0	(0)	0	(0)	0	(0)	1	(6)
11	0	(0)	10	(63)	0	(0)	0	(0)	0	(0)	0	(0)
12	–	–	–	–	0	(0)	1	(6)	0	(0)	3	(19)
Sum	0	–	13	–	1	–	19	–	18	–	37	–
Mean ± SD[a]	–	0 ± 0	–	27 ± 31	–	2 ± 4	–	17 ± 17	–	29 ± 36	–	33 ± 32

[a] Only those subjects with at least one value above the detection limit included ($N = 3$ for IL-1β, $N = 7$ for IL-6, $N = 7$ for TNF-α)

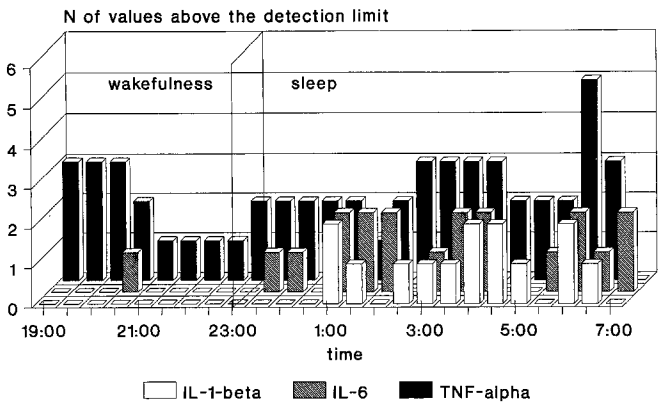

Fig. 1. Number of subjects with cytokine levels above the detection limit of the assays at the different time points across the 12-h period investigated

each cytokine value above the DL, whether it occurred during a NREM or a REM episode. Only those values were included, which occurred during a sleep cycle, which was not artificially terminated by the final awakening in the morning or by the end of polygraphic sleep recording. Ninety-four percent (15 out of 16) of IL-6 values and 70% (7 out of 10) of IL-1β values occurred during a NREM episode. However, as assessed by a binomial test, cytokine values above the DL were not more frequent during NREM episodes than would be expected on the basis of the average percentage of the duration of the NREM/REM cycles covered by the NREM episodes (79% and 78% for IL-6 and IL-1β, respectively).

Discussion

The present study demonstrates that in a considerable percentage of healthy subjects, carefully screened to be free of acute or chronic infectious disease, small amounts of IL-1β, IL-6 or TNF-α are released at night. IL-1β and IL-6 release occurred almost exclusively during sleep, but it was not related specifically to REM or NREM episodes. However, we could not detect a sleep-associated release of TNF-α.

Our results do not allow any precise quantification of sleep-associated cytokine release, because the plasma levels measured all were near to the detection limits of the assays. Therefore, these values have to be interpreted with caution, and some of them even may represent false positive ones. Nonetheless, our observation of a sleep-associated release of IL-1β and IL-6 seems to be valid, because false positive values should occur at random during a 12-h period of serial measurements.

To our knowledge, this is the first report on nocturnal plasma levels of IL-6 and TNF-α in humans, whereas enhanced IL-1-like activity during sleep in the plasma of healthy volunteers (Moldofsky et al., 1986) and increased IL-1β levels in the cerebrospinal fluid of sleeping cats (Lue et al., 1988) have already been described. The latter findings, together with the well-established knowledge that some cytokines are somnogenic, led to the hypothesis that cytokines are involved in the physiological regulation of sleep and wakefulness (Krueger 1990). This view is not supported unambiguously by the results presented here. We found sleep-related IL-1β release in only 27% and IL-6 release in only 58% of the subjects investigated; sleep-associated release of both cytokines in the same subject could only be documented in 17% of the volunteers. One may assume that in some subjects the amounts of cytokines released during sleep are too small to be detected by the assays used. In addition, because IL-1 is locally elaborated in the brain (Fontana et al. 1982; Breder et al. 1988), plasma levels may only poorly reflect sleep-associated increases in cytokine levels in the CNS. Nontheless, it is puzzling that we found the release of IL-6, which does not promote sleep in animals (Opp et al. 1989), to be sleep-associated, whereas we could not document a sleep-related release of TNF-α, which is somnogenic in rabbits (Shoham et al. 1987).

The assumption that cytokines are involved in physiological sleep regulation is not the only way to explain their sleep-associated release. It has been proposed that sleep plays a role in nonspecific host defense (Krueger and Johannsen 1989). This view is as well in line with the somnogenic activity of cytokines and it is supported by two additional lines of evidence. First, in rabbits inoculated with *Staphylococcus aureus*, prognosis is best in those animals showing the largest increase in NREM sleep (Toth and Krueger 1988). Secondly, sleep deprivation abrogates the clearance of influenza viruses from lung homogenates of previously immunized mice (Brown et al. 1989). Therefore, it is tempting to speculate that sleep-associated cytokine release in healthy subjects reflects an activation of host defense mechanisms, which is induced specifically during sleep. This activation may occur either independently from exogenic stimulation or in response to minor stimuli of the host response accumulated during prior wakefulness. A sleep-dependent activation of host defense mechanisms does, however, not preclude that cytokines are involved in physiological sleep regulation. Sleep and host defense may be linked bidirectionally.

Before, however, definite conclusions regarding nocturnal cytokine release and its functional significance can be drawn, two additional questions have to be answered. First, it would be important to know, whether nocturnal cytokine release is an intra-individually stable phenomenon, but until now no data are available to answer this question. Secondly, it is not known at present whether nocturnal cytokine release reflects a circadian rhythmicity, or whether it is truly sleep-dependent. The well-known nocturnal increase in cortisol, for example, is temporally associated with night-sleep under normal environmental conditions. It persists, however, during sleep deprivation (Davidson et al. 1991), which indicates that it is mainly governed by a circadian rhythm. Growth hormone (GH) is mainly released around sleep onset. In contrast to cortisol, GH secretion is suppressed by sleep deprivation (Davidson et al. 1991) and postponed by a delay of sleep onset (Born et al. 1988) indicating that it depends on sleep itself or on sleep inducing mechanisms rather than on a circadian pacemaker. With

respect to cytokines, the only study reporting nocturnal levels during sleep deprivation remains inconclusive, because Moldofsky et al. (1989) reported that IL-2-like activity was highest around the usual time of sleep onset, whereas IL-1-like activity was not.

We conclude that small amounts of IL-1β, IL-6 and TNF-α are released at night in a considerable percentage of healthy subjects. The release of IL-1β and IL-6 is temporally associated with sleep, whereas the release of TNF-α is not. It is not possible at present to decide whether sleep-associated cytokine release reflects an interaction between sleep and host defense mechanisms or a sleep-independent circadian rhythmicity.

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