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Effects of an interleukin-1 receptor antagonist on human sleep, sleep-associated memory consolidation, and blood monocytes

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ABSTRACT

Pro-inflammatory cytokines like interleukin-1 beta (IL-1) are major players in the interaction between the immune system and the central nervous system. Various animal studies report a sleep-promoting effect of IL-1 leading to enhanced slow wave sleep (SWS). Moreover, this cytokine was shown to affect hippocampus-dependent memory. However, the role of IL-1 in human sleep and memory is not yet understood. We administered the synthetic IL-1 receptor antagonist anakinra (IL-1ra) in healthy humans (100 mg, subcutaneously, before sleep; $n = 16$) to investigate the role of IL-1 signaling in sleep regulation and sleep-dependent declarative memory consolidation. Inasmuch monocytes have been considered a model for central nervous microglia, we monitored cytokine production in classical and non-classical blood monocytes to gain clues about how central nervous effects of IL-1ra are conveyed. Contrary to our expectation, IL-1ra increased EEG slow wave activity during SWS and non-rapid eye movement (Non-REM) sleep, indicating a deepening of sleep, while sleep-associated memory consolidation remained unchanged. Moreover, IL-1ra slightly increased prolactin and reduced cortisol levels during sleep. Production of IL-1 by classical monocytes was diminished after IL-1ra. The discrepancy to findings in animal studies might reflect species differences and underlines the importance of studying cytokine effects in humans.

1. Introduction

Sleep and the immune system are thought to interact such that sleep promotes immune defense and immune responses to infectious agents convey an enhancing influence on sleep (Besedovsky et al., 2012; Imeri and Opp, 2009). Stimulated by early work that identified a muramyl peptide, i.e., a fragment of bacterial cell walls, as potent sleep promoting factor (Krueger et al., 1982), the idea has been around for a long time that pro-inflammatory cytokines that are released in response to microbial challenge are involved in the homeostatic regulation of sleep, especially of slow wave sleep (SWS) (Krueger and Majde, 1994; Krueger et al., 1995). Interleukin-1 beta (IL-1) and tumor necrosis factor (TNF), two important cytokines mediating inflammatory processes, are considered major players in this context (Krueger et al., 2007). Intracerebroventricular (i.c.v.) injection of IL-1 and TNF enhances non-rapid eye movement (NonREM) sleep duration and slow wave activity in rats, mice and rabbits, whereas inhibition of IL-1 signaling diminishes Non-REM sleep in these species (Krueger et al., 2007; Obál and Krueger, 2003). Whereas initially these cytokines were assumed to originate from peripheral sites, more recent research focused on brain microglia and macrophages as crucial source of IL-1 and TNF, both of which are released during synaptic activity and promote SWS also under normal physiological conditions, i.e., in the absence of any infectious challenge (Krueger et al., 2008). Apart from regulating sleep, microglial pro-inflammatory cytokines are involved in plastic neuronal processes underlying memory formation in the hippocampus (Williamson et al., 2011). Both IL-1 and TNF contribute to synaptic long-term potentiation (LTP), a major mechanism underlying the formation of neuronal memory representations (Ben Menachem-Zidon et al., 2011; del Rey et al., 2013; Gruber-Schoffnegger et al., 2013; Schneider et al., 1998).

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Whereas in animals administration of IL-1 and TNF induced robust enhancements of NonREM sleep with opposite effects after blocking respective receptors (e.g., Opp and Krueger, 1991; Takahashi et al., 1996b, 1997), there is an obvious paucity of studies of such cytokines in humans. Minocycline which directly suppresses microglial activation in an anti-inflammatory manner, decreased SWS and NonREM sleep in humans and animals, respectively (Nonaka et al., 1983; Wisor et al., 2011). In pilot studies, patients with rheumatoid arthritis and obstructive sleep apnea showed a decrease in sleepiness after blocking of IL-1 and TNF-signaling, respectively (Omdal and Gunnarsson, 2005; Vgontzas et al., 2004). In another study, abstinent patients with alcohol dependence showed a robust decrease in REM sleep following treatment with the TNF antagonist etanercept (Irwin et al., 2009). Surprisingly, in these human studies TNF blockade did not alter polysomnographic signs of NonREM sleep (Vgontzas et al., 2004; Irwin et al., 2009), which contrasts with findings in rabbits, where i.c.v. injection of a TNF receptor blocker mainly diminished NonREM sleep (Takahashi et al., 1996b). Although primary effects of pro-inflammatory cytokines on NonREM and SWS have been demonstrated also in humans (e.g., Raison et al., 2010; Späth-Schwalbe et al., 2000), to the best of our knowledge so far no attempts have been made to specifically scrutinize the role of IL-1 for human SWS.

Here, we investigated the effects of s.c. administration of a single dose of the IL-1 receptor antagonist anakinra (IL-1ra) on sleep in healthy humans. As the antagonist crosses the blood–brain barrier (Cawthorne et al., 2011; Clark et al., 2008; Gutierrez et al., 1994), we expected to replicate the suppressive effects on SWS observed after i.c.v. administration of similar IL-1 antagonists in animals (Opp and Krueger, 1991; Takahashi et al., 1997). Considering the well-known consolidating effect of SWS on hippocampus-dependent declarative memory (Diekelmann and Born, 2010) as well as findings that microglial-derived pro-inflammatory cytokines contribute to hippocampal LTP, we also investigated effects of IL-1ra on overnight consolidation of declarative memory (word-pairs, texts). Finally, to gain hints on how central nervous effects of IL-1ra are mediated, we monitored IL-1 production by circulating monocytes, in parallel with sleep and overnight memory consolidation. In this context, we aimed to elucidate which monocyte subpopulation (i.e., either $CD14^{++}CD16^{-}$ or $CD14^{dim}CD16^{++}$ monocytes) can be used as a suitable model of brain microglial IL-1 production, accessible to evaluation in human blood (Anthony et al., 2005; Leone et al., 2006; Varvel et al., 2012).

2. Material and methods

2.1. Subjects

Subjects were 16 healthy men aged on average 22.75 ± 0.93 years (range 18–31 years). Women were not included in this study because of known interactions between sleep and the menstrual cycle (e.g., Baker and Driver, 2007) and, thus, to reduce inter-individual variance, although this limits generalizability of results. Subject numbers were calculated by power analysis based on results from other pharmacological studies using comparable outcome measures (e.g., Feld et al., 2014; Groch et al., 2013). They were non-smokers presenting a normal nocturnal sleep pattern and did not take any medication at the time of the experiments. Acute and chronic illness was excluded by medical history, physical examination, and clinical routine laboratory investigation. The subjects had a regular sleep–wake rhythm for at least 6 weeks before the experiments. Subjects were familiarized with the experimental setting by spending an adaptation night in the laboratory that included the attachment of electrodes for sleep recordings and the insertion of an intravenous (i.v.) forearm catheter for blood sampling. Written informed consent was obtained from each participant, and the study was approved by the local ethics committee.

2.2. Experimental design and procedure

The experiments were conducted in the sleep laboratories of the University Hospital Schleswig–Holstein, Campus Luebeck, Germany, according to a placebo-controlled within-subject crossover design. Each man participated in two experimental conditions. In one condition he was subcutaneously (s.c.) injected with the IL-1ra anakinra (Kineret®, Biovitrum, Sweden; 100 mg dissolved in 0.67 ml saline solution), half-life in plasma 4–7 h (Akash et al., 2013; Chang et al., 2004); in the other condition placebo (saline solution that was visually indistinguishable from anakinra) was injected. Participants, examiners and people assessing the outcomes of the measurements were all blinded to the condition. The order of substance administration was balanced across subjects, who were randomly allocated to one of the two possible orders based on the automated calculation of a random number. Both sessions for a subject were separated by an interval of at least 14 days. The lack of differences in baseline values of IL-1ra between conditions proved that 2 weeks were sufficient for the drug being washed out, although this does not exclude that (undetected) biological effects might have persisted for a much longer time after the substance has been cleared from the body.

On experimental nights, participants arrived at the laboratory at 19:30 h. Following preparations for polysomnographic recordings, they performed (between 21:00 and 22:30 h, always in the same order) on a word pair associates learning task and a text learning task, with a 10-min break between the tasks. After the learning phase and 30 min before lights were turned off (at 23:00 h) to enable sleep, the substance was administered. Blood samples were taken at 20:30, 21:30, 22:30, 23:30 h, and then every hour until 6:30 h for analyses of hormone and IL-1ra concentrations and for flow cytometry. Blood was sampled via an i.v. forearm catheter that was connected to a long thin tube and enabled blood collection from an adjacent room without disturbing the subject's sleep. The total volume of blood sampled during a session was 250 ml.

Subjects were awakened at 7:00 h and then left the lab. During the following day participants engaged in their usual activities. They were instructed to refrain from any stressful mental or physical activities, which were confirmed by a record of their activities during the day. In the evening they returned to the lab at 20:00 h and retrieval of the memory tasks was tested – in reverse order of learning and a last blood sample was taken. Possible side effects of IL-1ra administration were excluded by a standardized interview and questionnaire assessing drug awareness and symptoms like dizziness, pain, headache, etc. on the morning after sleep and after subjects returned to the lab in the evening. There were no significant side effects.

2.3. Polysomnography, sleep EEG analyses, and subjective sleep quality

Standard polysomnographic recordings were obtained including electroencephalographic (EEG) recordings from electrodes attached at C3 and C4 (according to the international 10–20 system, referenced to electrodes attached to the mastoids) as well as electrooculographic and electromyographic recordings. Signals were amplified (Brain Amp, Brain Products, Germany) and digitized, with the EEG sampled at a rate of 200 Hz and filtered between 0.16 and 70 Hz. Sleep stages were determined off-line for subsequent 30-s recording epochs following standard criteria (Rechtschaffen and Kales, 1968). Total sleep time (TST), and the time spent in the different sleep stages (wake; stages 1, 2, 3, and

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