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IL-6-trans-signalling increases rapid-eye-movement sleep in rats

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Abstract

Interleukin 6 (IL-6), a cytokine of the gp130-signalling-family, plays an important role in immediate immunological functions, in metabolism and in the central nervous system. IL-6-signalling is mediated by classic-signalling via the membrane bound IL-6 receptor or by IL-6-trans-signalling via the soluble IL-6 receptor. Whereas the receptor subunit gp130 is ubiquitously expressed within the body, IL-6 receptor expression is restricted to distinct cell populations. Within the brain parenchyma the IL-6 receptor is sparsely expressed, and therefore the brain is mostly dependent on IL-6-trans-signalling in its response to IL-6. Recently we have shown that IL-6-trans-signalling but not classic-signalling plays a pivotal role in the establishment and maintenance of chronic inflammation and cancer, whereas its role in sleep regulation has not been studied so far. We reasoned that the IL-6-trans-signalling mimetic Hyper-IL-6 which in contrast to IL-6 alone can activate almost all cells of the brain might have a profound effect on sleep regulation and performed sleep recordings with rats injected with recombinant Hyper-IL-6. In the present study, the i.c.v. administration of the designer cytokine Hyper-IL-6 into rats at dark onset increased the amount of rapid-eye-movement sleep (REM sleep) but did not affect non-rapid-eye-movement sleep (non-REM sleep). Our data define a new role of IL-6-trans-signalling in sleep regulation.

Keywords

IL-6-trans-signalling; Sleep; Hyper-IL-6; Cytokine; (Rat)

1. Introduction

Beyond the function of interleukin 6 (IL-6) in immunological processes (Rose-John and Neurath, 2004), IL-6 is involved in the development and maintenance of the central nervous system (Balschun et al., 2004). IL-6 is integrated into the regulation of the hypothalamic–

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pituitary–adrenal axis. Together with leptin and insulin-like growth factor (IGF) IL-6 plays a central role in the regulation of the metabolic balance of sugar- and fat metabolism (Matarese and La Cava, 2004). Furthermore it has some impact on the regulation of synaptic long-term potentiation and promotes plasticity (Balschun et al., 2004). In a dose dependent manner IL-6 has somnogenic and pyrogenic effects (Hogan et al., 2003; Opp et al., 1989; Vgontzas et al., 2003). Many other cytokines in addition to IL-6 are important in sleep regulation and/or homeostasis, e.g. IL-1 β , TNF α , IL-4, IL-10, IL-18; most of these impact IL-6 expression (for review: Krueger et al., 2007).

Although there is strong evidence that IL-6 is involved in sleep regulation (Hogan et al., 2003; Späth-Schwalbe et al., 1998), the results from previous animal studies showed conflicting results with regard to the effect of exogenous manipulations of the IL-6 system. Whereas one group found that central (i.c.v.) and peripheral (i.p.) injection of recombinant human IL-6 into rabbits did not alter non-rapid-eye-movement sleep (non-REM sleep) and rapid-eye-movement sleep (REM sleep) (Opp et al., 1989), other researchers saw that i.c.v. administration of recombinant rat IL-6 to rats led to an initial increase and subsequent suppression of non-REM sleep with no changes in REM sleep. Antagonizing exogenous IL-6 via anti-IL-6 antibodies had no effect on sleep (Hogan et al., 2003). Interestingly, IL-6 deficient mice displayed no differences in non-REM sleep, but increased REM sleep (Morrow and Opp, 2005). However, there is also evidence that the regulation of IL-6 signalling is modulated by sleep (Dimitrov et al., 2006; Redwine et al., 2000; Vgontzas et al., 2004).

There are two signalling pathways that can be activated by IL-6. In classic-IL-6-signalling, IL-6 first binds to the membrane bound interleukin 6 α -receptor (IL-6 receptor; gp80). Thereupon the IL-6/IL-6 receptor complex associates with the β -receptor gp130 leading to gp130-homodimer formation and signal transduction. A naturally occurring soluble IL-6 receptor has a similar affinity to IL-6 as the membrane bound IL-6 receptor. Complexed with IL-6 the soluble IL-6 receptor can stimulate cells, which only express gp130. Such cells, lacking the membrane bound IL-6 receptor could normally not respond to IL-6. This trans-signalling process is important for the regulation of cellular differentiation and apoptosis and the development and maintenance of chronic inflammation and cancer (see review: Scheller et al., 2006). IL-6-trans-signalling can be mimicked by the designer cytokine Hyper-IL-6, which is a fusion protein of human IL-6 and the human soluble IL-6 receptor connected by a flexible peptide linker (Fischer et al., 1997). Hyper-IL-6 but not IL-6 has been demonstrated to be highly effective on neuronal cells (März et al., 1997b; Schäfer et al., 1999). The human Hyper-IL-6 was previously shown to be active on rat cells in primary cultures of astrocytes (März et al., 1999a).

By using IL-6 for stimulations or injections it is not possible to discriminate between the classic and trans-signalling pathway. Therefore we investigated the effect of the trans-signalling mimetic Hyper-IL-6 after i.c.v. application on sleep–wake behaviour in rats. We asked whether Hyper-IL-6, which — in contrast to IL-6 — can activate all cells of the brain, has an effect on sleep architecture.

2. Material and methods

Hyper-IL-6 was expressed and purified as previously described (Fischer et al., 1997) and was able to induce specifically IL-6-trans-signalling (Supplementary Fig. 1 A–C). The specificity of the recombinant protein Hyper-IL-6 was demonstrated in several cell-based and biochemical assays (Supplementary Fig. 2 A–D). Male Sprague–Dawley rats weighing 300–380 g at the time of surgery were used. Rats were kept individually in Plexiglas cages in sound-attenuated, temperature-regulated environmental chambers at 23 ± 1 °C on a 12-h light:12-h dark cycle (light on at 5:00 a.m.). Water and food were available *ad libitum*. Institutional guidelines for the care and use of research animals were followed and approved by the Institutional Animal Care and Use Committee.

For surgery, the rats were anesthetized by intraperitoneal (i.p.) injection of ketamine and xylazine mixture (87 and 13 mg/kg, respectively). Stainless steel screw electrodes for electroencephalographic (EEG) recordings were implanted over the frontal and parietal cortices and electromyographic (EMG) electrodes were placed into the dorsal neck muscle. An intracerebroventricular (i.c.v.) cannula (PlasticOne, 22 gauge) was inserted into the right lateral ventricle by using stereotaxic equipment [coordinates: 0.80 mm posterior, 1.8 mm lateral to the bregma, and 4.5 mm ventral from the surface of the skull, according to the rat brain atlas by Paxinos and Watson (2005)]. The size of the internal cannula was 30 gauge and extended 0.5 mm beyond the tip of the guide cannula. The guide cannula and the screws were fixed to the skull with dental cement. The correct i.c.v. cannula placement was determined by the gravity method (sudden drop in pressure) during implantation and 3–4 days after surgery by the drinking response to i.c.v. injection of 200 ng angiotensin II (Epstein et al., 1970). Only rats responding to angiotensin were used for experiments. After surgery the rats were allowed at least 7–10 days to recover.

At least 3 days before experiments of sleep and wake recording, the rats were connected to the recording cables for adaptation to the experimental conditions. The recording cables, plugged to communicators, were connected to amplifiers. The signals of EEG and EMG were converted from analog to digital at a sampling rate of 128 Hz and collected by computers. The EEG was filtered below 0.5 Hz and above 30 Hz. EMG activity aided to determine the vigilance state.

The experimental procedures were as followed. Rats were injected i. c.v. with 2 µl pyrogen-free isotonic NaCl (PFS) prior dark onset following 23-h sleep–wake recording as baseline. The next experimental day the rats received an i.c.v. injection prior dark onset of either 100 ng human Hyper-IL-6 ($n=4$) or 500 ng Hyper-IL-6 ($n=4$), in a volume of 2 µl. EEG and EMG signal recording started immediately after injections.

The states of vigilance were visually scored off-line in 10-s epochs and classified as non-REM sleep, REM sleep or wakefulness according to the criteria of Neckelmann and Ursin (1993). The amount of time spent in each vigilance state was calculated for 2-h time blocks. The number of transitions from one vigilance to another served as indicator of sleep fragmentation.

For spectral analysis, EEG of each artefact-free 10-s epoch scored as non-REM or REM sleep was subjected to a fast Fourier Transformation (FFT) analysis, yielding power spectra between 0.5 and 20 Hz with a 0.5 Hz resolution (Sleep Sign). EEG power in REM and non-REM sleep were expressed as percentage of individual maximum EEG power in non-REM sleep to correct for individual differences in the absolute power (Franken et al., 1998).

For comparison of duration of each vigilance state and numbers of transitions between the baseline and experimental day, two-way analyses of variance (ANOVA) for repeated measures (factors: treatment and time effect) were calculated. An α -level of $P < 0.05$ was considered to be significant. As post-hoc analysis the Tukey test was used. The same statistical analysis method was used for comparison of EEG power spectra (factors: treatment and frequency effect).

For temperature measurement an e-mitter without batteries (Vital View®) was surgically implanted into the peritoneal cavity. For temperature recording the animal cage was placed onto a receiver plate (Vital View®) and the signal was routed to a computer.

3. Results

After Hyper-IL-6 exposure a modified behaviour of the rats was not observed visually within the EEG-recording time. The body core temperature did not change significantly after i.c.v. injection of 500 ng Hyper-IL-6 (data not shown).

There was a clear circadian rhythm in the sleep–wake behaviour determined from EEG signals, after cytokine or saline injection. This is reflected in the highly significant time effects within the recording time (Fig. 1; ANOVA, time effects, $P < 0.0001$). After i.c.v. administration of 100 ng Hyper-IL-6 into rats, sleep–wake behaviour did not change in terms of Wake (Fig. 1A), non-REM sleep (Fig. 1C), REM sleep (Fig. 1E) and transitions between the states (Fig. 1G). I.c.v. injection of 500 ng Hyper-IL-6 did not alter the amounts of Wake (Fig. 1B) and non-REM sleep (Fig. 1D), but significantly increased the amount of REM sleep (Fig. 1F; ANOVA, treatment effect, $F(1,33)=7.559$, $P=0.0096$; interaction “treatment×time”, $F(10,33)=2.015$, not significant). Post-hoc performed multiple pairwise comparisons of treatments (Tukey test) for each 2-h time blocks revealed statistical differences for the postinjection time points 18 and 20 h during the light period. There was no statistical significant increase of transitions between sleep states after the application of 500 ng Hyper-IL-6 (Fig. 1H; ANOVA, treatment effect, $F(1,36)=2.907$, $P=0.0968$; interaction “treatment×time”, $F(11,36)=0.8848$, not significant).

Analysis of the dark period at the second day (24–34 h postinjection) after 500 ng Hyper-IL-6 injection revealed no statistical difference to saline treatment of the first dark period (first 12 h postinjection) for amounts of non-REM sleep, REM sleep and transitions between states (data not shown).

A spectral analysis of EEG signals (Fig. 2A and B) displayed no effect of Hyper-IL-6 (500 ng) on EEG spectra for non-REM sleep (Fig. 2A). Also for REM sleep no shift in theta peak frequency was present but a significantly reduced relative power density after 500 ng Hyper-IL-6 application (Fig. 2B; ANOVA, treatment effect, $F(3,126)=17.09$, $P < 0.0001$; frequency

effect, $F(20,126)=6.173$, $P<0.0001$, interaction “treatment×frequency”, $F(60,126)=0.7095$, not significant).

4. Discussion

This is the first study to systematically investigate the influence of IL-6-trans-signalling on sleep regulation. Hyper-IL-6, that mimics the IL-6-trans-signalling pathway, had no impact on non-REM sleep in rats. Although i.c.v. injected rat IL-6 in high concentrations (400 ng) led to slightly increased non-REM sleep with a fragmentation of sleep, the amount of non-REM sleep is not altered by antagonizing IL-6 (Hogan et al., 2003) as well as by the lack of IL-6 in IL-6 knock-out mice (Morrow and Opp, 2005). Therefore Morrow and Opp (2005) hypothesised that IL-6 may not be involved in regulation or modulation of non-REM sleep. This hypothesis is supported by our experiments. Interestingly, Hyper-IL-6 application (i.c.v., 500 ng) had a quantitative (increased amount) and qualitative (reduced EEG power density) effect on REM sleep. An increase in REM sleep duration occurred predominately during the light period. This delayed effect of Hyper-IL-6 after 18–20 h after injection could be probably because of an indirect sleep promoting effect of Hyper-IL-6, including activation of brain cells (astrocytes, glia cells, neurons) mediating the observed REM sleep increase. This effect was somewhat surprising since IL-6 deficient mice show a 30% increase in REM sleep, mainly during the light period (Morrow and Opp, 2005). However, these seemingly contradictory findings might be explained by the different properties of IL-6-classic and trans-signalling. Hyper-IL-6 can activate a broader spectrum of target cells within the brain and has been shown to be far more potent than IL-6. For the activation by IL-6 the IL-6 receptor in membrane bound or soluble form is required. Whereas the receptor subunit gp130 is ubiquitously expressed within the body and also in most of the brain areas (for gp130 protein expression in rat brain see Watanabe et al., 1996), the expression of the IL-6 receptor is restricted to distinct cell populations, e.g. hepatocytes and lymphocytes. Within the rat brain, IL-6 receptor mRNA expression has been found in some neuronal lineages, like primary sympathetic neurons (März et al., 1996, 1998), in hypothalamus, neocortex, cerebellum (Gadiant and Otten, 1993) and rarely in several other brain structures (Yan et al., 1992). In addition, low levels of IL-6 receptor mRNA were detected in microglia and astrocytes, but not in oligodendrocytes of primary mixed brain cultures of newborn mice (Sawada et al., 1993).

However, it should be kept in mind that mRNA expression might not reflect protein expression onto the cell surface. Recently, Helwig et al. (2008) analysed rat brain slices via immunohistochemistry and found IL-6 receptor protein expression in cells of the third ventricle but not within the brain parenchyma. The ependymal cells of the ventricles are activated after i.c.v. application of IL-6 (Vallieres et al., 1997). Thus IL-6 floating within brain ventricles could signal through the membrane bound IL-6 receptor via the classic transduction pathway. Indeed, it has been shown that many neural cells, e.g. primary rat sympathetic neurons and newborn rat astrocytes, are only responsive to IL-6 in the presence of the soluble IL-6 receptor or by Hyper-IL-6 via trans-signalling (März et al., 1998, 1999a,b; Schöbitz et al., 1995). All these findings seem to indicate that IL-6 activity within the brain parenchyma requires the trans-signalling pathway. Interestingly, in healthy humans a sleep-associated increase of the soluble IL-6 receptor was found (Dimitrov et al., 2006). In

pathological conditions, IL-6, which is mainly released by astrocytes (Van Wagoner et al., 1999), and the soluble IL-6 receptor, increase (Klein et al., 1995; März et al., 1997a; Montero-Julian, 2001). During disease processes an altered sleep behaviour is well documented (as short selection: Dantzer, 2004; De Cock et al., 2008; Fang et al., 1995; Toth and Krueger, 1988), and thus a link to trans-signalling seems very likely. Moreover, expression and activity of the metalloprotease ADAM17 (Edwards et al., 2008), which is responsible for the generation of the soluble IL-6 receptor, increases under some pathological conditions including stroke. These processes promote neural progenitor cell migration and contribute to stroke-induced neurogenesis (Moro et al., 2003) and might thereby also contribute to altered sleep behaviour during disease conditions.

Regardless, single cytokines never act alone but in a network associated with other cytokines, hormones and cellular molecules. In this context, our data define a REM sleep promoting role of IL-6-trans-signalling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi: 10.1016/j.ejphar.2009.04.023.

Fig. 1.

Effects of i.c.v. injection of 100 ng and 500 ng recombinant Hyper-IL-6 (closed circles, thick lines) and pyrogen-free isotonic saline (open circles, thin lines) into rats on Wake (A, B), non-REM sleep (C, D), REM sleep (E, F) and transition of states (G, H). Values are mean $\pm$ S.E.M. ($n=4$). Asterisks denote significant treatment effect. Horizontal black bars indicate the dark period.

Fig. 2.

Effect of i.c.v. injection of 500 ng Hyper-IL-6 (red line) and pyrogen-free isotonic saline (green line) into rats on EEG power spectra obtained from non-REM (A) and REM epochs (theta peak frequency, B) shown for light (dashed line) and dark period (solid line) respectively. Values are mean of four animals. The black bar with the asterisk indicate a significant treatment effect (ANOVA). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)