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Interleukin 1 receptor contributes to methamphetamine- and sleep deprivation-induced hypersomnolence

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Abstract

Methamphetamine-induced wakefulness is dependent on monoamine transporter blockade. Subsequent to methamphetamine-induced wakefulness, the amount of time spent asleep and the depth of sleep are increased relative to baseline sleep. The mechanisms that drive methamphetamine-induced hypersomnolence are not fully understood. We recently observed that methamphetamine exposure elevates the expression of the sleep-promoting cytokine, interleukin-1 β in CD11b-positive monocytes within the brain. Here, we sought to determine whether activation of the interleukin 1 receptor (IL1R) drives the increase in the depth and amount of sleep that occurs subsequent to methamphetamine-induced wakefulness. IL1R-deficient mice and wild type control mice were subjected to systemic methamphetamine (1 and 2mg/kg) and saline treatments. The wake-promoting effect of methamphetamine was modestly potentiated by IL1R-deficiency. Additionally, the increase in time spent in NREMS subsequent to methamphetamine-induced wakefulness in wild type mice was abolished in IL1R-deficient mice. The increase in time spent asleep after 3 h of behaviorally enforced wakefulness was also abolished in IL1R-deficient mice. Increases in EEG slow wave activity triggered by methamphetamine and sleep deprivation were of equal magnitude in IL1R-deficient and wild type mice. These data demonstrate that IL1R activation contributes to hypersomnolence that occurs after sleep loss, whether that sleep loss is triggered pharmacologically by methamphetamine or through behavioral sleep deprivation.

Keywords

Amphetamines; Cytokines; Psychostimulants; Sleep homeostasis; Slow waves; EEG

1. Introduction

Methamphetamine (METH) has profound effects on sleep/wake cycles. Exposure to METH results acutely in sustained wakefulness, an effect that is dependent on blockade of the cell membrane dopamine transporter [17]. Hypersomnolence occurs after METH-induced waking in rats [16] and during METH withdrawal in humans [11]. The mechanisms

underlying hypersomnolence subsequent to METH-induced wakefulness are not certain. In previous work [18], cells of the monocyte lineage (i.e., CD11b-positive cells) were isolated from other brain cell types in vitro by immunoaffinity. According to real-time polymerase chain reaction assays, IL1 β mRNA was upregulated by METH relative to saline only in this CD11b-positive cell pool. Several lines of evidence indicate that IL1 β is a sleep-promoting substance (reviewed in [6]). For instance, intracerebroventricular administration of IL1 β promotes sleep [1], whereas antagonism of IL1 β by intracerebroventricular antibody administration suppresses sleep [12].

The elevation of IL1 β expression at the mRNA level by METH exposure [18] led us to hypothesize that IL1 β mediates the hypersomnolence that occurs after METH administration. We therefore sought to determine whether METH-induced hypersomnolence is dependent on IL1R activation. IL1R-deficient mice and wild type controls were subjected to intraperitoneal administration of METH at wake promoting doses of 1 and 2 mg/kg. Electroencephalographic and electromyographic measurements were taken in the 8-h interval immediately subsequent to administration.

2. Materials and methods

Experiments adhered to the National Research Council Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Washington State University. Ten IL1R-deficient (IL1R KO) male mice (B6.129S7-Il1r1tm1Imx/J strain; JAX #3245) and ten wild type coisogenic males (C57BL/6J; JAX # 664) were purchased from Jackson Laboratories at age 7 weeks. Mice were subjected to surgical implantation of fronto-parietal EEG and nuchal EMG leads (as in [15]) under isoflurane anesthesia, during which one IL1R KO mouse died due to experimenter error. Data were amplified and digitized by the Pinnacle Technologies 8200 system (Lawrence, KS) as described elsewhere [15]. State classification in 10-s epochs and spectral analysis for quantification of NREMS delta power (EEG power in the 0.5–4 Hz range) were performed with Neuroscore 2.01 software (Data Sciences Inc., St. Paul, MN) as described elsewhere [15]. Average NREMS delta power was calculated from the first 90 epochs of sleep subsequent to treatment onset.

2.1. Experiment 1: effects of acute METH exposure on sleep

Mice were subjected to a dose response curve for measurement of the acute wake-promoting effects of METH. Mice were placed in the recording environment and tethered to the recording preamplifier at the end of the light phase of the LD12:12 cycle. On each of the next three days, one of the three treatments (intraperitoneal saline, METH 1 mg/kg and METH 2 mg/kg) was administered 5 h into the daily light phase (ZT5). The order of treatments was randomized individually for each mouse. These doses of METH are modeled after previous studies on the wake-promoting effect of METH in mice [17,18] and do not produce overt neurotoxicity in rodents [3]. EEG and EMG data were collected for an interval of 8 h after treatment.

2.2. Experiment 2: comparison of hypersomnolence responses after sleep deprivation (SD) and METH 2 mg/kg

We sought to determine whether IL1R was necessary for SD induced sleep loss in addition to METH-induced sleep loss. Mice previously subjected to METH treatments were subjected to 3 h of SD, enforced by continuous rotation of a metal bar beneath the bedding in the floor of the cage, as described previously [15]. The bar rotated continuously at a frequency of roughly 0.1 Hz. Therefore, the bedding beneath the animal was disrupted once every 5 s by the bar. The intention was to induce an amount of sleep loss equivalent to the loss incurred by METH at 2 mg/kg, as measured in experiment 1. The duration of waking induced by METH at 2 mg/kg was slightly greater than 3 h. We expected that the animals would remain awake transiently after termination of SD, and therefore terminated SD at 3 h under the assumption that the duration of waking would be equivalent to that produced by METH 2 mg/kg. Mice were allowed to sleep spontaneously for a period of 5 h after termination of SD, during which time their EEG and EMG signals were collected for analysis.

2.3. Statistics

Statistics were performed with Statistica 9.0 software (Statsoft, Inc.). For experiment 1, dependent variables were subjected to repeated measures analysis of variance (ANOVA) with genotype as a grouping factor and dose of methamphetamine (0, 1, or 2 mg/kg) as a within subjects factor. For experiment 2, dependent variables were subjected to repeated measures analysis of variance (ANOVA) with genotype as a grouping factor and treatment (saline vs. methamphetamine 2 mg/kg vs. SD) as a within subjects factor. When ANOVA yielded a significant main effect or dose $\times$ genotype interaction, Student's *T* with Bonferroni correction was performed as a post hoc planned contrast. Reported measures of variability are standard error of the mean (SEM).

3. Results

3.1. METH-induced wakefulness is not attenuated by IL1R deficiency

METH caused a dose-proportionate increase in time spent awake. Mice of both genotypes were awake more than 95% of the first 2 h after METH 1 mg/kg and more than 95% of the first 3 h after METH 2 mg/kg. ANOVA with hourly interval and METH dose as within subjects factors yielded a significant effect of METH on time spent awake ($F_{2,34} = 143.1$, $P < 0.001$), time spent in NREMS ($F_{2,34} = 140.2$, $P < 0.001$), and time spent in REMS ($F_{2,34} = 61.1$, $P < 0.001$). All of these effects were also significant when data were collapsed into two 4-h bins for analysis (Fig. 1; all panels $P < 0.001$ for main effect of METH). Time spent awake was significantly increased in mice of both genotypes during the 4-h interval immediately after administration of METH 1 or 2 mg/kg relative to saline (Fig. 1A), while time spent in NREMS (Fig. 1C) and REMS (Fig. 1E) was significantly reduced in the 4-h interval immediately after administration of METH 1 or 2 mg/kg relative to saline treatment. Comparisons across genotype groups did not indicate a significant difference between genotypes in time spent awake, in NREMS, or in REMS during the 4-h interval immediately after administration. A complementary measure, the latency to sleep onset after administration, confirmed that the wake-promoting efficacy of METH was not attenuated by

IL1R KO (Fig. 2A). ANOVA yielded a robust effect of dose on the latency to sleep onset ($F_{2,34} = 273.16$, $P < 0.001$) but no effect of genotype ($P = 0.665$) or genotype $\times$ treatment interaction ($P = 0.363$) on latency to sleep onset.

3.2. Recovery sleep after METH-induced sleep loss is attenuated by IL1R-deficiency

Despite the strong suppression of sleep by METH in both genotypes, ANOVA yielded a significant effect of genotype on NREMS ($F_{1,17} = 4.80$, $P = 0.043$) across the entire 8-h post-treatment recording session. This effect reflects the fact that wild type mice, but not IL1R KO mice, underwent hypersomnolence subsequent to METH-induced wakefulness. In hours 5–8 post-treatment, wild type mice exhibited increased time spent in NREMS subsequent to METH 2 mg/kg relative to saline treatment. This 20% increase in time spent in NREMS (106 min vs. 86 min) was significant in wild type mice (Fig. 1D). The 5% increase in NREMS in IL1R KO mice (94min vs. 89min) in hours 5–8 after METH 2mg/kg (Fig. 1D) was not significant. Increased NREMS in wild type mice in hours 5–8 after METH (2 mg/kg) came at the expense of wake, which was reduced significantly by 16% relative to saline in this interval (115 min vs. 134min; Fig. 1B). In IL1R KO mice, time spent awake in hours 5–8 post-treatment was not significantly reduced after METH 2 mg/kg relative to saline (Fig. 1B). REMS in the same time interval was unaffected by treatment in either genotype (Fig. 1F).

Effects of METH on other measures of NREMS homeostatic drive were modulated inconsistently by IL1R-deficiency. The amount of time lapsed between administration of METH 1 mg/kg and the accumulation of 1 h of time asleep was greater by 14% in IL1R KO mice (270 min) than in wild type controls (236 min; Fig. 2B). Since the amount of time lapsed between administration and sleep onset did not differ between genotypes at this or any other dose (Fig. 2A), the difference between genotypes in the response to METH at 1 mg/kg in Fig. 2B was a function of a delay, in IL1R KO mice, in the accumulation of sleep after the wake-promoting effect of METH had terminated. ANOVA yielded a significant dose $\times$ genotype interaction in affecting the amount of time lapsed between sleep onset and 1 h of accumulated sleep (dose $\times$ genotype interaction $F_{2,34} = 5.97$, $P = 0.006$). IL1R KO mice exhibited a 41% greater value for this measure (124 ± 10 min) relative to wild type (87 ± 6 min) after METH 1 mg/kg, indicative of sleep fragmentation in IL1R KO mice. The number of brief awakenings from sleep was significantly affected by genotype (main effect of genotype $F_{1,17} = 6.23$, $P = 0.023$). A greater number of awakenings from sleep in IL1RKO mice (28 ± 3) relative to wild type mice (19 ± 2) irrespective of METH dose may, therefore, have contributed to the delay in the accumulation of time asleep in IL1R KO mice relative to wild type mice.

In contrast to sleep timing variables, EEG slow wave activity (SWA; EEG power in the 1–4 Hz range) was not modulated by genotype. When EEG power spectra were measured in 1 Hz bins across treatments, ANOVA yielded a significant frequency $\times$ dose interaction ($F_{38,646} = 21.13$, $P < 0.001$) in affecting EEG power spectra, indicating that there was a frequency-specific modulation of EEG power by METH. NREMS SWA was calculated as a planned comparison, and was greater after both doses of METH relative to saline in both strains of mice (main effect of treatment $F_{2,34} = 29.18$, $P < 0.001$). This effect of METH was

not significantly modulated by genotype. Thus, while the timing of sleep after METH treatment and the occurrence of brief awakenings are dependent on IL1R, changes in EEG SWA are not.

3.3. Recovery sleep after enforced wakefulness is attenuated by IL1R-deficiency

Mice of both genotypes were subjected to a 3-h SD session. The duration of SD was intended to match METH 2 mg/kg in the magnitude of sleep loss. However, the two treatments were not identical in this measure: repeated measures ANOVA yielded a significant effect of treatment (METH 2 mg/kg vs. 3-h SD; $F_{1,17} = 13.78$, $P = 0.002$), though not genotype ($P = 0.076$), on latency to sleep. Relative to SD, METH incurred an excess of 22 min of sleep loss in wild type mice and an excess of 29 min of sleep loss in knockout mice, representing 12 and 15% differences between treatments in the two genotypes (Fig. 2A). By contrast, there was a significant effect of genotype on the latency to accumulation of 1 h of sleep after METH 2 mg/kg and 3-h SD (main effect of genotype $F_{1,17} = 4.91$, $P = 0.041$), but no effect of treatment on this measure. Additional measures of hypersomnolence that were, as shown above, impacted by METH (wakefulness in hours 5–8 post-treatment, NREMS in hours 5–8 post-treatment, NREMS EEG SWA) were affected in an analogous manner by 3-h SD (Table 1). Wake as a percentage of time was significantly reduced after 3-h SD in wild type mice but not IL1R KO mice. NREMS as a percentage of time was significantly elevated after 3-h SD in wild type mice but not IL1R KO mice. Therefore, IL1R KO attenuates the drive to recover sleep whether the sleep loss is incurred as a consequence of METH exposure or behaviorally enforced wakefulness.

4. Discussion

IL1R is activated by IL1 β , a known sleep regulatory molecule [1,5,6,7,8]. We show that this signaling mechanism must be intact in order for hypersomnolence to occur subsequent to METH-induced wakefulness. This effect of IL1R-deficiency does not, however, reflect a role for the receptor that is unique to the response to METH-induced sleep loss. The increase in NREMS as a percentage of time after SD-induced sleep loss was also attenuated in IL1R KO mice relative to wild type mice. These data demonstrate a general function for IL1R in the detection of, and recovery from, sleep loss. They complement a previous study [2], which demonstrated attenuated responses to sleep loss in dual IL1R/tumor necrosis factor receptor 1-deficient mice; the current study shows that this phenotype is produced by deficiency for IL1R alone.

Our previous work demonstrated an increase in IL1 β transcript levels in cerebral monocytes (i.e., CD11b-positive cells) after METH 2mg/kg [18]. Intracerebroventricular infusion of IL1 β increases time spent in NREMS [1]. This effect of IL1 β is dependent on IL1R [4]. Conversely, blocking this pathway in the brain by intracerebroventricular infusion of anti-IL1 β antibodies increases time spent awake [12]. There is a significant increase in IL1 β mRNA concentration in hypothalamus, hippocampus, cerebral cortex and brainstem of rats after 6-h SD [13]. IL1 β decreases the magnitude of excitatory postsynaptic potentials produced by electrical stimulation of neuronal circuits and slightly increases the magnitude of inhibitory postsynaptic currents [9]. Collectively, these data demonstrate an essential function for increased cerebral IL1 β concentration and IL1R activation in hypersomnolence

subsequent to sustained wakefulness. Given the similar effects of IL1R-deficiency on SD- and METH-induced hypersomnolence, it can only be concluded from the current study that hypersomnolence due to METH-induced waking is simply a function of IL1R-dependent homeostatic sleep regulation.

It is important to discriminate between two possible mechanisms by which METH may perturb sleep/wake cycles. Low non-toxic doses of METH, such as those used in the current study, trigger homeostatic, IL1R-dependent increases in time spent asleep that are not, as shown here, manifestly different from those caused by behavioral SD. Higher doses of amphetamines are neurotoxic, and are disruptive to a number of sleep regulatory circuits and to the production of sleep substances. For instance, the wake-promoting neuromodulators serotonin and dopamine are depleted in the brains of chronic METH users (reviewed in [19]). Exposure to amphetamine congeners increases IL1 β concentration in the brain even after drug clearance and protects against toxicity of higher doses of amphetamines in a manner that is dependent on IL1R signaling [10]. Blockade of IL1R signaling by icv infusion of soluble IL1R potentiates amphetamine-induced neurotoxicity [14]. Intracerebroventricular administration of IL1 β to drug naive mice is neuroprotective against subsequent amphetamine exposure [10]. These data indicate that increased IL1R activation serves a neuroprotective role in response to amphetamine exposure. But given the sleep-promoting effect of IL1R signaling, the neuroprotective elevation of IL1 β expression after amphetamine exposure would be expected to come at the cost of increased somnolence. A systematic study of the long-term effects of neurotoxic amphetamine doses on sleep in IL1R KO mice will be necessary to ascertain whether IL1 β signaling contributes to long-term effects of amphetamine exposure on sleep.

5. Conclusions

Collectively with previous observations that METH elevates IL1 β expression, and does so selectively in monocytes within the brain [18], the current data support the concept that monocytes contribute to the effects of METH on sleep/wake cycles.

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Abbreviations

ANOVA	analysis of variance
EEG	electroencephalogram
EPSP	excitatory postsynaptic potential
IL1β	interleukin 1 β
IL1R	interleukin 1 receptor

IL1R KO	interleukin 1 receptor-deficient (knockout)
METH	methamphetamine
NREMS	non-rapid eye movement sleep
REMS	rapid eye movement sleep
SD	sleep deprivation
SWA	slow wave activity

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Fig. 1.

Effects of methamphetamine on time spent awake, in NREMS and in REMS in wild type animals (black bars) and IL1R KO mice (white bars). Vigilance states are plotted as a percentage of time during hours 1–4 after treatment (A, C, E) and hours 5–8 after treatment (B, D, F). Asterisks indicate a significant effect of METH vs. saline in the same genotype. Triangles indicate a significant effect of METH 1 mg/kg vs. METH 2mg/kg in the same genotype (Student's *T* with Bonferroni correction).

Fig. 2.

Latencies to cumulative sleep milestones after METH and SD treatments. (A) Latency to the first epoch classified as sleep after administration of saline, METH 1 mg/kg, METH 2mg/kg and 3-h SD in wild type mice (black bars) and IL1R KO mice (white bars). (B) Latency to the accumulation of 1 h of sleep after administration of saline, METH 1 mg/kg, METH 2 mg/kg and 3-h SD. *, significantly different from saline, same genotype, Student's *T* with Bonferroni correction. §, significantly different from IL1R KO, same treatment. Triangles indicate a significant effect of METH 1 mg/kg vs. METH 2 mg/kg in the same genotype (Student's *T* with Bonferroni correction).

Table 1

Effects of methamphetamine 2mg/kg and 3-h sleep deprivation on hypersomnolence measures.

Genotype	Wake (percent of time)			Main effect of treatment <i>F</i> =3.93; <i>P</i> =0.029
	Saline	METH 2mg/kg	3-h SD	
Wild type	57 ± 2	48 ± 3 *	52 ± 2 *	
IL1R KO	55 ± 3	54 ± 2	58 ± 2	
Genotype	NREMS (percent of time)			Main effect of treatment <i>F</i> =4.50; <i>P</i> =0.018
	Saline	METH 2mg/kg	3-h SD	
Wild type	36 ± 2	44 ± 2 *	41 ± 2 *	
IL1R KO	55 ± 3	39 ± 2	36 ± 2	
Genotype	NREMS EEG delta power (μV ² /Hz)			Main effect of treatment <i>F</i> = 49.39; <i>P</i> < 0.001
	Saline	METH2mg/kg	3-h SD	
Wild type	571 ± 56	687 ± 72 *	970 ± 90 *	
IL1R KO	501 ± 24	682 ± 45 *	979 ± 99 *	

* Indicate a significant effect of treatment vs. saline in the same genotype (Student's *T* with Bonferroni correction).