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Acute Cocaine Increases Interleukin-1 β mRNA and Immunoreactive Cells in the Cortex and Nucleus Accumbens

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Abstract

The cytokine, interleukin-1 β (IL1 β) is a sleep regulatory substance whose expression is enhanced in response to neuronal stimulation. In this study, IL1 β mRNA and immunoreactivity (IR) are evaluated after acute cocaine. First, IL1 β mRNA levels were measured at the start or end of the light period after saline or acute exposure to a low dose of cocaine (5 mg/kg, intraperitoneal (ip)). IL1 β mRNA levels after an acute exposure to cocaine (5 mg/kg, ip) at dark onset were significantly higher than those obtained from rats sacrificed after an acute exposure to saline in the piriform and somatosensory cortex, and nucleus accumbens. Acute exposure of cocaine at 5 mg/kg at dark onset also increased the number of IL1 β -immunoreactive astrocytes in layer I–V of the prefrontal cortex, somatosensory cortex and nucleus accumbens. These data suggest that IL1 β mRNA and protein levels in some of the dopaminergically innervated brain regions are responsive to cocaine.

Keywords

Use-dependent; Sleep; Cytokine; Interleukin 1 beta; Astrocyte; Cocaine-induced plasticity

Introduction

Psychostimulants, such as cocaine, can disrupt sleep architecture after repeated use as well as sleep-related cognitive learning [1]. Our overall hypothesis is that disruptions in sleep may play a role in altering the cocaine-induced neuroplasticity in the mesolimbic cortical regions of the brain [2]. By altering the neural circuits involved in natural motivation, psychostimulants may produce a spiraling down hill course into drug addiction. Cocaine enhances the levels of dopamine at the synapses in the motivational brain regions, such as the prefrontal cortex (PFC), nucleus accumbens (NAcc) and the amygdala. Another response to cocaine is an increase in cytokine or growth factor production, such as brain-derived neurotrophic factors [3]. This response of growth factors to cocaine may partially explain the alterations in neuroplasticity that occur with cocaine exposure.

Cocaine affects the immune system and alters the levels of proinflammatory cytokines [4–6]. Cocaine alters sympathetic balance and reduces the innate immune responses [4]. In the brain, the central beta adrenergic receptors are involved in the activation of the cytokine, interleukin-1 β (IL1 β), in the hypothalamus by methamphetamine [7]. In the brain, cytokines act as sleep regulatory substances (SRSs) that are produced in response to neural activity [8–10]. Whisker stimulation increases the number of cytokine-IR cells in the somatosensory cortex. In particular, the number of IL1 β -IR astrocytes is increased in the cortical layers that receive whisker stimulation.

Other stressors also activate IL-1 β within the brain [11–16]. Footshock stress increases IL1 β in the hypothalamus; this effect is attenuated by blocking the norepinephrine (NE) receptor and enhanced by blocking the NE transporter [11]. Since pharmacological action of cocaine may involve blockade of the NE transporter as well as the dopamine (DA) and serotonin (5HT) transporters [17], then acute cocaine would be expected to enhance IL1 β production within the brain.

Our specific hypothesis is that sleep functions to modulate neuronal connectivity in response to use-dependent adaptations that occur during the wake state. The molecular basis for adaptations in neuronal connectivity is hypothesized to involve growth factors that promote sleep, such as the brain cytokines [18]. The brain cytokine network is implicated in the regulation of a variety of physiological and pathological processes [18–21]. For example, one of these cytokines, IL1 β , is involved in the homeostatic process of sleep regulation [18, 22]. Administration of exogenous IL1 β enhances sleep or sleepiness when given to cats, rabbits, rats, mice or humans. Inhibition of IL1 β inhibits spontaneous sleep. Inhibition of IL1 β attenuates the sleep rebound that occurs after sleep deprivation. Sleep deprivation is associated with an increase in IL1 β mRNA levels [23, 24] and IL1 β -like activity in the cerebrospinal fluid of cats varies with sleep cycle [25]. A diurnal variation of IL1 β mRNA levels occurs in many brain regions in the rat [23, 26]. IL1 β mRNA levels at light onset were significantly higher than those obtained from rats sacrificed just prior to dark onset in the PFC, somatosensory and amygdala/piriform cortex, ventral hippocampus, hypothalamus, nucleus tractus solitarius, and NAcc, but not in the dorsal hippocampus.

In this study our objective is to investigate the role of IL1 β in response to an acute exposure to cocaine. Initially we chose a low dose of cocaine (5 mg/kg, ip) to investigate the response of IL1 β mRNA levels just after light onset (start of the sleep period in rats) or just prior to dark onset (start of the wake period in rats). Therefore we have evaluated the impact of cocaine on IL1 β mRNA levels in the PFC and NAcc, as well as brain regions important in sleep regulation, such as the hypothalamus and other cortical regions such as the somatosensory or piriform cortex, as a function of time of day. Subsequent to these studies, we evaluated in a second group of rats whether an acute exposure to cocaine would alter the number of IL1 β -immunoreactive cells in the PFC, NAcc, piriform and somatosensory cortex just after dark onset. We evaluated the immunoreactive cells to determine if the changes observed in the IL1 β mRNA levels after dark onset were expressed in protein levels as well.

Experimental procedures

Male Sprague–Dawley rats weighing 250–280 g were acclimatized to their cages for 7 days prior to experimentation. Animals were housed in groups of 2–4 and fed standard rat chow and water ad libitum. The rats were handled by the experimenter that would inject them for three days prior to the injection to acclimate the rats to the handling process. The room was maintained on a 12 h light:dark cycle with the lights on at 00:00 h. Care of the animals was in accordance with NIH standards published in the Guide for Care and Use of Laboratory Animals and was approved by our Institutional Animal Care and Use Committee.

Injections

The first group of rats ($n = 6$ for each group) was injected acutely in the intraperitoneal cavity with saline or cocaine (5 mg/kg) at light onset (00:00) or 1 h prior to dark onset (11:00). These rats were killed 1 h later for mRNA analyses since the mRNA levels are responsive at shorter time intervals. These brains were quickly removed, dissected as previously described and frozen in liquid nitrogen [27]. The second group of rats used for immunohistochemistry was injected with saline or 5 mg/kg cocaine just after dark onset (12:00) and then placed into the locomotor activity chambers. At 14:00, the rats were anesthetized with isoflurane and cardiac-perfused with warm 0.9% saline followed by cold 4% paraformaldehyde in phosphate buffered saline. The brains were removed, postfixed in 4% paraformaldehyde, sunk in 20% sucrose overnight, frozen in crushed dry ice and stored at -80°C until sectioned for immunohistochemistry. The rats used for immunohistochemistry were sacrificed at 2 h after the injections since changes in the protein levels follow upregulation in the mRNA levels.

RT–PCR

After thawing the dissected tissue, each brain region was individually homogenized using a tissue tearer in 2 ml of TRIzol (Invitrogen; Carlsbad, CA) at 4°C and the RNA was purified according to the manufacturer's instructions. After isopropanol precipitation, the RNA pellet was hydrated in 50 μl DNase RNase Free water (Gibco; Grand Island, NY). The samples were then treated with DNase I (Ambion; Austin, TX) according to the manufacturer's instructions. All samples were run on a gel and the quantity of RNA was determined by absorbance reading at optical density (OD) of 260 nm prior to real time RT–PCR. Firststrand cDNA was synthesized by priming with oligo-dT using 1 μg total RNA. Real time RT–PCR

was performed using the ThermoScript RT system (Invitrogen). Aliquots (5 μ l) of a 1:20 dilution of cDNA (12 ng of total RNA) were amplified by real time RT-PCR. The primers for IL1 β were 5'-ACCCAAGCACCTTCTTTTCC'-3' and 5'-AGACAGCACGAGGCATTTTT-3' and for cyclophilin were 5'-CTTTGCAGACGCCGCTGTCTC-3' and 5'-ACGCTCCATGGCTTCCACAAT-3''. These primers were designed for rats using NCBI primer BLAST. The primer efficiencies for IL1 β and cyclophilin were greater than 95%. Real time RT-PCR was performed using an iCycler IQ multi-color real time RT-PCR detection system (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's instructions. Reactions were performed in a 25 μ l volume with 5 μ l of the sample run in quadruplicate. Platinum[®] qPCR Supermix-UDG (Invitrogen), SYBR Green I mix (Molecular Probes; Eugene, OR) and ROX (Bio-Rad) were added. The IL1 β protocol consisted of 5 cycles at 94°C for 15 s, 60°C for 15 s, and 62°C for 15 s, then 38 cycles at 94°C for 15 s, 58°C for 15 s, and 72°C for 15 s.

Statistical Analyses for RT-PCR

For all groups, the data were analyzed with the iCycler iQ software and by using the statistical program NCSS. All the reactions of the samples were performed in quadruplicate, and each C_t value was an average of the values obtained from each reaction. The C_t values were determined by subtracting the cyclophilin C_t values from the gene of interest C_t values. The control saline at dark onset's average was then subtracted from all the C_t values to obtain C_t values. The relative fold changes were obtained by taking the 2^{-C_t} of this number. Two-way ANOVAs were used to compare daytime and nighttime values (time) and the saline vs. cocaine values (treatment). When the interaction was significant (below 0.05), then the saline vs. cocaine values were compared at light or dark onset separately.

Immunohistochemistry

After treatment, immunohistochemistry was performed as previously described [9, 28]. These brains were sectioned coronally with a sliding microtome at 30 μ m thickness and sections collected for each antibody at intervals of 240 μ m. Tissue sections were washed in between each treatment with 3 washes of PBS (15 min each): (1) 50% alcohol for 30 min (to enhance antibody penetration), (2) 0.3% hydrogen peroxide in PBS (to block endogenous peroxidases) for 30 min, 3% normal horse serum (NHS) for 1 h at room temperature to block nonspecific binding. The sections were then incubated in a polyclonal anti-rat IL1 β antibody produced in goat (0.5 μ g/ml, catalog #AF501-NA, R&D Systems, Inc, Minneapolis, MN) with 2% NHS in PBS for 3 days at 4°C with gentle rocking. After incubation the sections were incubated in biotinylated antibody to goat IgG (1:500; Vector Labs, Burlingame, CA) with 2% NHS for horse anti-goat in PBS for 2 h at room temperature. Then the sections were incubated in an avidin-biotinperoxidase reagent (ABC kit at 1:200; Vector Labs, Burlingame, CA) for 90 min at room temperature. Finally the sections were developed in 0.04% diaminobenzidine tetrahydrochloride, 0.06% nickel chloride, and 15 μ l H₂O₂ in 10 ml buffer (DAB kit; Vector Labs). Negative controls for the IL1 β antibodies were performed by preincubating the primary antibody with an excess of the recombinant rat IL1 β , respectively. Previously, IL1 β knockout mice were evaluated to

verify that the IL1 β -IR astrocytes were specific to wild-type mice only [9]. No specific IR-labeling was observed in the control sections, verifying the specificity of the antibody.

Statistical Analyses for Immunohistochemistry

Since the immunohistochemical analyses were performed in pairs of saline- or cocaine-treated rats, then the paired Students' *t* test was used to evaluate the pairs of control and experimental groups.

Results

IL1 β mRNA Levels After Acute Cocaine

After control saline injections, the relative levels of IL1 β mRNA were 2–3 fold higher at 01:00 than at 12:00, in the PFC (time- $F[1, 19] = 30.18$, $P = 0.00003$), piriform ($F[1, 19] = 13.56$, $P = 0.0016$), and somatosensory ($F[1, 19] = 29.84$, $P = 0.00003$) cortex, hypothalamus ($F[1, 17] = 33.32$, $P = 0.00002$) and NAcc ($F[1, 19] = 59.73$, $P = 0.00000$) (Table 1). However, after an acute dose of cocaine (5 mg/kg, ip), the relative levels of IL1 β mRNA were significantly higher at 12:00 for the NAcc ($F[1, 19] = 17.15$, $P = 0.001$), somatosensory cortex ($F[1, 19] = 13.96$, $P = 0.001$) and hypothalamus ($F[1, 17] = 8.64$, $P = 0.009$) in comparison with those rats receiving saline (Table 1). For the piriform cortex ($F[1, 19] = 5.41$, $P = 0.031$) and hypothalamus ($F[1, 17] = 6.88$, $P = 0.018$), there was a significant interaction. In the piriform cortex, the rats receiving the cocaine treatment had a significantly greater level of IL1 β mRNA at the 12:00 time than for the rats receiving saline. In the hypothalamus, the relative levels of IL1 β mRNA were significantly reduced at 01:00 after cocaine in comparison with the rats exposed to saline but no differences were observed between saline and cocaine at 12:00.

IL1 β -IR cells after acute cocaine

Acute cocaine exposure at 12:00 significantly increased at 14:00 the number of IL1 β -IR astrocytes in layers I ($P = 0.0047$), II ($P = 0.005$), and V ($P = 0.002$) of the PFC (Fig. 1) and most of the layers of the somatosensory cortex (II–III- $P = 0.04$, IV- $P = 0.11$; V- $P = 0.01$ & VIP- $P = 0.01$) (Fig. 2) and the NAcc core ($P = 0.029$) and shell ($P = 0.001$) (Fig. 3; Table 2). No significant differences in the number of IL1 β -IR astrocytes were observed in the layers I–III of the piriform cortex ($P = 0.384$ and $P = 0.306$). There was a large variation in the number of IL1 β -IR astrocytes between different sections in both the saline- and cocaine-exposed rats.

Discussion

Current results confirm previous findings using real time RT-PCR that a diurnal rhythm in IL-1 β mRNA is expressed in many brain regions [23, 26]. In this study, we extended those observations by demonstrating that an acute exposure to a low dose of cocaine stimulates the dark onset levels of IL-1 β mRNA in the somatosensory and piriform cortex as well as the NAcc, but not in the main center for autonomic regulation, the hypothalamus. The lack of response of IL-1 β to cocaine at light onset may be due to the fact that the high levels of IL-1 β mRNA at this time have reached their maximal levels. In response to activity during

the night, IL-1 β -IR cells are greater in number at light onset than at dark onset [10]. At dark onset, the low dose of cocaine also significantly increased the number of IL1 β -IR astrocytes in the PFC, NAcc and somatosensory cortices, but not in the piriform cortex. The differences between the IL1 β mRNA and protein levels in response to cocaine in the prefrontal cortex may be due to the differences between evaluating a dissected region and the more precise evaluation available with the immunohistochemistry. Since the PFC and NAcc have been implicated in the expression of cocaine-induced plasticity [2], then the activity-dependent increase in this cytokine protein at dark onset suggest that this growth factor may be able to influence cocaine-induced plasticity.

A diurnal rhythm in the behavioral response to cocaine has also been observed with cocaine-induced conditioned place preference [29] and sensitization [30] studies. The rats show a greater sensitization and conditioned place preference to cocaine near dark onset than during the light period.

In this study, we evaluated the response of IL1 β to an acute dose of cocaine as a first step in evaluating molecular changes in sleep regulatory substances in response to drugs of abuse. These acute changes may set the stage for the long-lasting modifications that lead to drug addiction. However, other investigators have observed that the activity marker, cfos, and the brain-derived neurotrophic factor, BDNF, increased acutely to cocaine [31–33] but these increases were temporary.

IL1 β and other cytokines mediate numerous physiological functions such as sleep, feeding, development and body temperature [reviewed in 18–21, 34]. IL1 β is also implicated in learning; for instance, administration of IL1 β into the hippocampus impairs learning [35]. Pre-treatment on hippocampal slices with IL1 β blocks induction of long-term potentiation in the dentate gyrus [36]. Also long term potentiation is impaired in IL1-knockout mice [37]. IL1 β and other cytokines also play a role during pathology when their levels are amplified [reviewed in 34]. That there are variations in the levels of IL1 β mRNAs in brain regions implicated in cocaine-induced plasticity in response to cocaine is consistent with the hypotheses implicating IL1 β in neural plasticity.

The issue of how cocaine-induced activity induces expression of cytokines is not yet fully understood. One hypothesis is that extracellular ATP, released during glutamate neurotransmission, acts on purine type 2 receptors on glial cells [38], that in turn increases cytokine release [22, 39, 40]. For IL1 β , the mechanism seems to involve ATP-activation of caspase-1 in inflammasomes [41]. Caspase-1 in turn cleaves pro-IL1 β releasing the mature 17 kD form of IL1 β . Inflammasomes occur constitutively in brain [42, 43]. Other cytokines such as the neurotrophins have also been observed in pro-forms that are released in an activity-dependent fashion [44, 45]. Since the inhibition of the release of adenosine from astrocytes can modulate sleep homeostasis and its cognitive consequences in the brain [46], then the cocaine-induced changes in the IL1 β -IR astrocytes may also play a role in cocaine-induced plasticity.

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Fig. 1. IL1 β -IR astrocytes in layer I–III of the PFC 2 h after saline injection (*left*) or exposure to cocaine at 5 mg/kg (*right*). More IL1 β -IR astrocytes (*arrows*) are evident after the cocaine exposure. Scale bar = 0.075 mm

Fig. 2. IL1 β -IR astrocytes in layer II–III of the somatosensory cortex (Sctx) 2 h after saline injection (*left*) or exposure to cocaine at 5 mg/kg (*right*). More IL1 β -IR astrocytes (*arrows*) are evident after the cocaine exposure. Scale bar = 0.075 mm

Fig. 3. IL1 β -IR astrocytes in the nucleus accumbens (NAcc) 2 h after saline injection (*left*) or exposure to cocaine at 5 mg/kg (*right*). More IL1 β -IR astrocytes (*arrows*) are evident after the cocaine exposure. Scale bar = 0.075 mm

Table 1

Relative change in IL1 β mRNA levels in the prefrontal cortex (PFC), nucleus accumbens (NAcc), hypothalamus, or somatosensory or piriform cortex just after light onset (01:00) relative to dark onset (12:00) values 1 h after saline or cocaine (5 mg/kg, i.p.)

Brain regions	Treatment	Light onset	Dark onset
PFC	Saline	2.8 $\pm$ 0.4[*]	1.0 $\pm$ 0.1
	Cocaine-5	2.6 $\pm$ 0.1[*]	1.7 $\pm$ 0.2
NAcc	Saline	2.3 $\pm$ 0.1[*]	1.1 $\pm$ 0.2
	Cocaine-5	2.7 $\pm$ 0.1[*]	1.9 $\pm$ 0.1[†]
Hypothalamus	Saline	2.5 $\pm$ 0.2[*]	1.1 $\pm$ 0.2
	Cocaine-5	1.5 $\pm$ 0.2^{*†}	1.0 $\pm$ 0.1
Somatosensory Ctx	Saline	1.9 $\pm$ 0.2[*]	1.0 $\pm$ 0.1
	Cocaine-5	2.3 $\pm$ 0.04[*]	1.7 $\pm$ 0.1[†]
Piriform cortex	Saline	2.6 $\pm$ 0.4[*]	1.0 $\pm$ 0.1
	Cocaine-5	2.1 $\pm$ 0.3	1.7 $\pm$ 0.1[†]

^{*} $P < 0.05$, comparing light onset (1:00) and dark onset (12:00) with a two-way ANOVA

[†] $P < 0.05$, comparing Saline and Cocaine with a two-way ANOVA

Table 2

The number of IL1 β -IR astrocytes in each of the PFC, somatosensory cortex (Sctx) or piriform (Pir) cortex layers or the NAcc core or shell analyzed for the rats 2 h after saline or cocaine (5 mg/kg, ip) injected at 12:00

Layers	Saline	Cocaine-5 mg/kg
PFC-I (5)	38.7 $\pm$ 6.0	56.6 $\pm$ 6.7 *
PFC-II (5)	20.8 $\pm$ 3.2	31.5 $\pm$ 3.7 *
PFC-V (5)	21.5 $\pm$ 2.4	36.1 $\pm$ 3.0 *
Sctx-II–III (5)	6.8 $\pm$ 2.0	17.9 $\pm$ 3.6 *
Sctx-IV (5)	5.8 $\pm$ 2.2	10.6 $\pm$ 1.9
Sctx-V (5)	4.2 $\pm$ 1.5	12.9 $\pm$ 2.1 *
Sctx-VI (5)	5.1 $\pm$ 0.8	12.9 $\pm$ 2.4 *
NAcc core (4)	13.8 $\pm$ 1.1	20.8 $\pm$ 1.9 *
NAcc shell (4)	12.6 $\pm$ 1.0	22.0 $\pm$ 1.5 *
Pir ctx-I (5)	15.2 $\pm$ 2.6	13.9 $\pm$ 2.0
Pir ctx-II–III (5)	14.6 $\pm$ 1.1	13.0 $\pm$ 2.2

n = 4–5 for each treatment group as noted in parentheses

* $P < 0.05$, comparing Cocaine vs. Saline, using a Student's paired t test