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Endocannabinoid Modulation of Amphetamine Sensitization is Disrupted in a Rodent Model of Lesion-induced Dopamine Dysregulation

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Abstract

We tested the hypothesis that increased dopaminergic sensitivity induced by olfactory bulbectomy is mediated by dysregulation of endocannabinoid signaling. Bilateral olfactory bulbectomy induces behavioral and neurobiological symptomatology related to increased dopaminergic sensitivity. Rats underwent olfactory bulbectomy or sham operations and were assessed two weeks later in two tests of hyperdopaminergic responsivity: locomotor response to novelty and locomotor sensitization to amphetamine. Amphetamine (1 mg/kg i.p.) was administered to rats once daily for eight consecutive days to induce locomotor sensitization. URB597, an inhibitor of the anandamide hydrolyzing enzyme fatty-acid amide hydrolase (FAAH), was administered daily (0.3 mg/kg i.p.) to sham and olfactory bulbectomized (OBX) rats to investigate the impact of FAAH inhibition on locomotor sensitization to amphetamine. Pharmacological specificity was evaluated with the CB₁ antagonist/inverse agonist rimonabant (1 mg/kg i.p.). OBX rats exhibited heightened locomotor activity in response to exposure to either a novel open field or to amphetamine administration relative to sham-operated rats. URB597 produced a CB₁-mediated attenuation of amphetamine-induced locomotor sensitization in sham-operated rats. By contrast, URB597 failed to inhibit amphetamine sensitization in OBX rats. The present results demonstrate that enhanced endocannabinoid transmission attenuates development of amphetamine sensitization in intact animals but not in animals with OBX-induced dopaminergic dysfunction. Our data collectively suggest that the endocannabinoid system is compromised in olfactory bulbectomized rats.

Keywords

olfactory bulbectomy; endocannabinoid; amphetamine sensitization; FAAH; anandamide; dopamine

INTRODUCTION

Cannabinoid CB₁ receptors are widely distributed throughout the brain of several mammalian species, including rodents and humans (Herkenham et al., 1991; Herkenham et al., 1990). The best characterized endogenous ligands for this receptor are anandamide and 2-arachidonoylglycerol (2-AG) (Devane et al., 1992; Di Marzo et al., 1994; Stella et al., 1997). Endocannabinoids are synthesized and released on demand and then are rapidly deactivated by transport into cells followed by enzymatic hydrolysis (for review see Piomelli, 2005). Activation of presynaptic CB₁ receptors inhibits neurotransmission in a retrograde manner (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001).

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Endocannabinoids modulate both excitatory and inhibitory neurotransmission to control homeostasis throughout the central nervous system (Di Marzo and Petrosino, 2007).

The endocannabinoid system also modulates activity of the mesocorticolimbic dopamine system. CB₁ receptors are located in the striatum (Herkenham et al., 1991; Hohmann and Herkenham, 2000; Mailleux and Vanderhaeghen, 1992), where they reside presynaptically on glutamatergic afferents as well as GABAergic interneurons (Monory et al., 2007). In the striatum, cannabinoid CB₁ mRNA is localized to GABAergic medium spiny projection neurons as well as GABAergic interneurons (Herkenham et al., 1991; Hohmann and Herkenham, 2000; Mailleux and Vanderhaeghen, 1992). Postsynaptic endocannabinoid release is necessary for CB₁-mediated long-term depression of glutamatergic transmission in the striatum (Gerdeman et al., 2002). Thus, endocannabinoids may act as retrograde messengers to suppress cortico-striatal glutamate release onto striatal medium spiny neurons. Direct and indirect dopamine agonists also increase striatal anandamide levels (Centonze et al., 2004; Giuffrida et al., 1999). These findings suggest functional interactions between the endocannabinoid, glutamatergic and dopaminergic signaling systems.

We hypothesized that hyperdopaminergic dysfunction observed following olfactory bulbectomy is associated with impaired endocannabinoid signaling. Bilateral olfactory bulbectomy in the rodent induces heightened locomotor responsivity to a novel open field (i.e. novelty) (Klein and Brown, 1969; van Riezen and Leonard, 1990) and a “presensitized” locomotor response to indirect dopaminergic agonists such as cocaine (Chambers et al., 2004) and amphetamine (Gaddy and Neill, 1976). In addition, olfactory bulbectomized rats exhibit faster acquisition of amphetamine self-administration relative to sham rats (Holmes et al., 2002). Olfactory bulbectomy induces sprouting of dopaminergic axons in the ventral striatum, in which basal dopamine release and dopamine receptor levels are increased at behaviorally relevant time points (Gilad and Reis, 1979; Holmes, 1999; Lingham and Gottesfeld, 1986; Masini et al., 2004). The olfactory bulbectomized (OBX) rat thus serves as a model of dopaminergic hyperfunction.

Anandamide and its related bioactive congeners, the *N*-acylethanolamines (NAEs), are likely to be synthesized presynaptically by the enzyme *N*-acylphosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD). NAPE-PLD is confined to glutamatergic axon terminals, where the enzyme is found in association with intracellular calcium stores (Nyilas et al., 2008). Thus, the biosynthesis of anandamide may be related to the calcium-dependent state of glutamatergic axonal terminal. In the striatum, a portion of glutamatergic afferents are removed by olfactory bulbectomy (Kelly et al., 1997), suggesting that olfactory bulbectomy may also impair glutamate-dependent anandamide biosynthesis.

Anandamide is deactivated by the enzyme fatty-acid amide hydrolase (FAAH) (Cravatt et al., 1996, 2001; Devane et al., 1992). FAAH is localized to soma and dendrites of neurons that are postsynaptic to presynaptic CB₁ receptors (Egertová et al., 2003). Unlike direct agonists of CB₁ receptors, FAAH inhibitors do not produce unwanted psychoactive side-effects or profound motor impairment (Piomelli, 2005). The FAAH inhibitor URB597 produces CB₁-mediated anxiolytic effects (Kathuria et al., 2003; Moise et al., 2008; Patel and Hillard, 2006) as well as antidepressant (Bortolato et al., 2007) and anti-impulsivity (Marco et al., 2007) effects. Thus, URB597 represents a useful tool for studying the impact of FAAH inhibition on dopamine-dependent behaviors.

We used the olfactory bulbectomy model to investigate the role of endocannabinoids in modulating behaviors influenced by dopaminergic dysfunction – the locomotor response to novelty and sensitization to amphetamine. First, the olfactory bulbectomy model was validated by assessing locomotor activity in response to exposure to a novel open field in both sham-

operated and OBX rats. Next, locomotor sensitization to amphetamine was profiled in OBX and sham rats over eight consecutive days of amphetamine administration. The FAAH inhibitor URB597 was administered at a dose known to selectively increase the bioavailability of anandamide without altering levels of 2-arachidonoylglycerol in intact rats (Kathuria et al. 2003). We tested the hypothesis that FAAH inhibition would suppress amphetamine sensitization in sham-operated animals but not in animals in which a hyperdopaminergic state was induced by olfactory bulbectomy. The contribution of CB₁ receptors to URB597-mediated actions was evaluated using the competitive CB₁ antagonist/inverse agonist rimonabant.

MATERIALS AND METHODS

Subjects and Surgical Procedures

Male Sprague-Dawley rats ($N = 60$), Harlan, Indianapolis, IN) weighing approximately 250 – 300 g at surgery were used. All procedures were approved by the University of Georgia Animal Care and Use Committee. Rats were housed in groups of two to five in a humidity- and temperature-controlled animal housing facility. Lights in the colony room were on at 0600 and off at 1800. All behavioral testing was initiated during the light phase. Rats were randomly assigned to either sham or olfactory bulbectomy surgery. For olfactory bulbectomy surgery, rats ($n = 18$) were anesthetized with a combination of pentobarbital (65 mg/kg i.p.; Sigma, St. Louis, MO) and ketamine hydrochloride (100 mg/kg i.p.; Fort Dodge Laboratories, Fort Dodge, IA) or isoflurane (Abbott Laboratories, North Chicago, IL). Burr holes measuring 3 mm in diameter were bilaterally drilled approximately 5 mm anterior to bregma and 1 mm lateral to the midline. The dura mater was pierced and a curved plastic pipette tip was used to aspirate the olfactory bulbs. The resulting cavity was filled with Gelfoam (Upjohn, Kalamazoo, MI). Rats receiving the sham surgery ($n = 42$) underwent the same procedure except that the olfactory bulbs were not aspirated. Confirmation of olfactory bulb lesion was determined by brain dissection at the end of the experiment. Lesions were considered complete if the bulbs were completely severed from the forebrain, the weight of the tissue dissected from the olfactory bulb cavity did not exceed 5 mg, and frontal lobes were not bilaterally damaged. Histological verifications were performed by an experimenter blinded to the surgical condition.

Pharmacological Manipulations

URB597 was purchased from Cayman Chemical (Ann Arbor, MI). Rimonabant was a gift from NIDA. D-amphetamine sulfate was purchased from Sigma (St. Louis, MO). URB597 and rimonabant were dissolved in a 1:1:8 ratio of 100% ethanol:emulphor:saline. D-amphetamine sulfate (1 mg/kg) was dissolved in 0.9% saline. Drugs were administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. The FAAH inhibitor URB597 was administered at a dose (0.3 mg/kg i.p.) which selectively increases anandamide accumulation in intact rats, without altering levels of 2-AG (Kathuria et al., 2003). Rimonabant was administered at a dose (1 mg/kg i.p.) that blocks pharmacological effects of URB597, but does not exert locomotor or anxiogenic effects when administered alone (Jarbe et al., 2006; Moise et al., 2008; Patel and Hillard, 2006). Animals were randomly assigned to drug conditions that included ethanol: emulphor: saline vehicle (sham $n = 4$, OBX $n = 4$), saline (sham $n = 10$, OBX $n = 5$), URB597 (0.3 mg/kg i.p.) (sham $n = 13$, OBX $n = 9$), URB597 (0.3 mg/kg i.p.) coadministered with rimonabant (1 mg/kg) (sham $n = 8$), or rimonabant (1 mg/kg i.p.) alone (sham $n = 7$). All animals ($N = 60$) received amphetamine 15 min following these pharmacological manipulations.

Locomotor Sensitization to Amphetamine

Locomotor sensitization to amphetamine was assessed (see Figure 1 for diagrammed procedure) using a model similar to that validated previously using cocaine (Chambers et al., 2004). At least two weeks following surgery, rats were placed individually in the center of a

polycarbonate activity monitor chamber (Med Associates, St. Albans, VT) measuring 44.5 × 44 × 34 cm housed in a darkened, quiet room. A 25-watt bulb shone over the chamber. Activity was automatically measured by computerized analysis of photobeam interrupts (Med Associates). Total distance traveled in the arena was obtained from the computer program and used for data analysis. Rats remained undisturbed in this chamber for 30 min. At the end of this period, according to previously randomly assigned drug conditions, rats were injected i.p. with vehicle, saline, URB597 (0.3 mg/kg), URB597 (0.3 mg/kg) coadministered with rimonabant (1 mg/kg) or rimonabant (1 mg/kg) alone. Rats were then placed back in the center of the chamber and remained undisturbed for 15 min. Activity was again automatically recorded by the computer software. At the end of this period, rats were injected with d-amphetamine sulfate (1 mg/kg i.p.) and placed back into the chamber, undisturbed, for 45 min. Activity was automatically recorded by Med Associates computer software during the entire interval. Rats were subjected to the exact same procedure once daily for a total of eight consecutive days (see Figure 1).

Data Analysis

On each day of behavioral testing, distance traveled was recorded in three consecutive phases: (1) pre-injection open field activity (for 30 min), (2) pre-amphetamine open field activity determined after injection of vehicle/saline, URB597, URB597 coadministered with rimonabant or rimonabant alone (for 15 min), and (3) post-amphetamine open field activity (for 45 min), as diagrammed in Figure 1. Novelty-induced locomotor activity, measured during the first 30 min open field session on day 1, was analyzed with a between subjects (sham vs. OBX) Student's t-test. Differences in average distance traveled between surgical and drug groups were analyzed with repeated measures analysis of variance (ANOVA) with Fisher's Least Significant Difference post hoc tests. The source of significant interactions was explained, in the case of two group comparisons, using Student's t-tests. Friedman's test for nonparametric repeated measures ANOVA was used to analyze sensitization trends. "Distance traveled" counts obtained from activity meter software at various time points served as the dependent variable. In a small minority of cases (1% of data points or 43 out of 4320 data points), because of technical difficulties with computer software or the open field arena, data points were incomplete. Missing data values were therefore replaced with group means for that specific time point on that day. $P < 0.05$ was considered significant.

RESULTS

Control Conditions

In both sham and OBX groups, distance traveled post-amphetamine did not differ between saline (vehicle for amphetamine) and ethanol:emulphor: saline (vehicle for URB597 and rimonabant) vehicle-treated animals [$P > 0.05$ for both comparisons]. Therefore, saline-treated animals were combined with the ethanol:emulphor:saline vehicle-treated animals to form what will be referred to hereafter as the "vehicle"-treated control group.

Exposure to Novel Open Field (Day 1)

To verify that the OBX animals in our study exhibited characteristic hyperlocomotor responses to novelty, locomotor behavior elicited by exposure to a novel open field was measured in OBX and sham-operated rats. OBX animals exhibited 24.5 % greater locomotor activity than sham rats during the 30 min exposure to the novel open field arena ($t_{58} = 1.82$, $P < 0.05$, one-tailed) (see Figure 2), as described in other published reports (Klein and Brown, 1969; van Riezen and Leonard, 1990).

Repeated Exposure to Open Field (Day 2–7 Pre-injection Sessions)

To confirm that OBX rats behaved similarly to sham-operated rats when placed in an open field arena that was no longer novel, distance traveled was assessed on days 2–7 during the 30 min pre-injection sessions. Olfactory bulbectomy did not affect locomotor activity during this period. Locomotor activity declined similarly in both OBX and sham animals that received vehicle over consecutive days of testing ($F_{6,126} = 13.87$, $P = 0.001$). Moreover, cannabinoid pharmacological manipulations did not affect distance traveled in sham or OBX animals during the 30 min habituation session on days 2–7 ($P > 0.05$ for both analyses) (data not shown). Therefore, effects of URB597 and rimonabant on amphetamine sensitization (on day 2–7) did not depend upon locomotor responses elicited in response to the open field itself.

Cannabinoid Pharmacological Manipulations (Pre-amphetamine Sessions)

The impact of cannabinoid pharmacological manipulations on basal locomotor activity was evaluated in pre-amphetamine sessions (see Figure 1) to enable us to separate effects on basal locomotor activity from effects on locomotor sensitization to amphetamine. For this analysis, distance traveled during the 15 min pre-amphetamine interval was assessed over eight consecutive days of testing. Animals received once daily administration of either vehicle, URB597 (0.3 mg/kg i.p.), URB597 (0.3 mg/kg i.p.) coadministered with rimonabant (1 mg/kg i.p.), or rimonabant (1 mg/kg i.p.) alone. Olfactory bulbectomy did not affect distance traveled during this pre-amphetamine interval in animals that received vehicle ($P > 0.05$) (data not shown). The first and last four days of preamphetamine locomotor activity were analyzed separately to determine whether possible locomotor effects of URB597, observed during the preamphetamine session, could explain any effects of URB597 on locomotor sensitization to amphetamine (see post-amphetamine session results below). In sham animals, URB597 produced a modest but reliable decrease in distance traveled during the preamphetamine session during the first four days of testing ($F_{2,32} = 6.11$, $P < 0.01$). This effect was blocked by coadministration of rimonabant ($P < 0.05$ for both post hoc comparisons) (see Figure 3a). However, URB597 did not affect locomotor activity during the preamphetamine interval during the last four days of testing ($P > 0.05$) (see Figure 3b). This latter interval corresponds to the period during which URB597 suppressed locomotor sensitization to amphetamine in sham animals (see post-amphetamine session results below). Thus, effects of URB597 on amphetamine sensitization in our study cannot be attributed merely to acute locomotor effects of the FAAH inhibitor. In sham animals, rimonabant (1 mg/kg i.p.) did not alter distance traveled during the pre-amphetamine sessions relative to vehicle over either the first four ($P > 0.05$) or the last four ($P > 0.05$) days of the sensitization protocol (see Figure 3c, d). Similar conclusions were reached when locomotor activity was analyzed across the entire eight consecutive days of testing (data not shown). In OBX animals, URB597 did not alter distance traveled during the preamphetamine sessions relative to vehicle over either the first four ($P > 0.05$) or last four ($P > 0.05$) days of testing (see Figure 3e, f). Similar conclusions were reached when locomotor activity was assessed across all eight consecutive days of testing.

Amphetamine-induced Locomotor Activity (Post-amphetamine Sessions)

To determine the initial locomotor response to amphetamine, distance traveled during the first post-amphetamine session was compared in OBX and sham-operated rats (i.e. on day 1). On day 1, OBX rats receiving vehicle showed greater locomotor responses to amphetamine than sham-operated rats that similarly received vehicle ($F_{1,21} = 6.88$, $P < 0.05$) (see Figure 4a). Amphetamine-induced locomotor activation increased but then leveled out over the 45 min observation interval in both groups ($F_{4,84} = 27.36$, $P < 0.001$) (see Figure 4a). Olfactory bulbectomy differentially increased distance traveled over time on the first day of amphetamine administration ($F_{4,84} = 3.72$, $P < 0.01$); OBX animals traveled more than shams from 27–45 min post-amphetamine administration ($P < 0.05$ for all comparisons) (see Figure 4a).

To determine the impact of olfactory bulbectomy on amphetamine sensitization, amphetamine-induced distance traveled was compared in vehicle-treated OBX and sham-operated rats over all eight days of testing. A time-dependent sensitization to amphetamine developed in sham-operated groups ($F_{8,14}=32.64$, $P < 0.001$); distance traveled on days 5–8 was greater than that observed on day 1 ($P < 0.05$ for all comparisons, Dunn's multiple comparisons). By contrast, OBX animals did not further sensitize to locomotor effects of amphetamine ($P > 0.05$) (see Figure 4b).

Drug Effects on the Development of Amphetamine-induced Sensitization

OBX animals responded very differently to both amphetamine and URB597 relative to sham-operated rats. Therefore, the impact of cannabinoid pharmacological manipulations on amphetamine sensitization was evaluated in each surgical group separately. Sham animals that received vehicle sensitized to the locomotor effects of amphetamine; amphetamine-induced distance traveled was greater during the last four days of the sensitization protocol than during the first four ($F_{8,14} = 35.1$, $P < 0.0001$, Friedman's test). Distance traveled on day 8 was greater than that observed on days 1, 3 or 4. Amphetamine-induced locomotor activity was also greater on days 5–7 than on day 1 ($P < 0.05$ for all comparisons, Dunn's multiple comparisons) (see Figure 5a, b). Furthermore, in sham animals that received URB597, amphetamine-induced locomotor sensitization did not occur ($P > 0.05$, Friedman's). Therefore, effects of cannabinoid pharmacological manipulations were analyzed during the first four and last four days of the sensitization protocol separately to best describe the impact of URB597 treatment on the development of amphetamine sensitization.

In sham animals, amphetamine-induced distance traveled was not affected by cannabinoid pharmacological manipulations during the first four days of testing ($P > 0.05$) (see Figure 5a). Pharmacological manipulations did, however, alter amphetamine-induced distance traveled during the last four days of the sensitization protocol ($F_{2,32} = 3.72$, $P < 0.05$). Shams that received URB597 traveled less than those that received vehicle and this effect was blocked by rimonabant ($P < 0.05$ for both post hoc comparisons) (see Figure 5b). In sham animals, locomotor sensitization to repeated amphetamine administration was observed during the last four days of testing ($F_{3,96} = 5.73$, $P = 0.001$) (see Figure 5b). In sham animals, rimonabant alone did not affect the development of amphetamine sensitization relative to vehicle during either the first ($P > 0.05$) or last four ($P > 0.05$) days of testing (see Figure 5c, d). Amphetamine-induced locomotor sensitization was similarly increased in sham-operated groups receiving either vehicle or rimonabant. Identical conclusions were reached when distance traveled was analyzed across the first four ($F_{3,57} = 3.48$, $P < 0.05$) or the last four ($F_{3,57} = 3.44$, $P < 0.05$) days of the sensitization protocol (see Figure 5c, d) or across all 8 consecutive days of testing.

In OBX animals, amphetamine-induced distance traveled was similar across all 8 days of amphetamine administration ($P > 0.05$), reflecting the lack of further sensitization to amphetamine in OBX animals (see Figure 5e, f). Furthermore, URB597 did not alter the development of amphetamine sensitization in OBX rats during either the first ($P > 0.05$) or last four ($P > 0.05$) days of the sensitization protocol (see Figure 5e, f).

DISCUSSION

The olfactory bulbectomized rat is a model in which dopaminergic transmission is profoundly altered. OBX rats exhibit increases in dopamine-dependent behaviors such as an increased behavioral sensitivity to novelty (Klein and Brown, 1969; van Riesen and Leonard, 1990) and drugs of abuse (Gaddy and Neill, 1976; Holmes et al., 2002; Chambers et al., 2004). Olfactory bulbectomy also increases dopaminergic activity in the ventral striatum (Gilad and Reis, 1979; Holmes, 1999; Lingham and Gottesfeld, 1986; Masini et al., 2004). The olfactory bulbectomy model was therefore employed to examine the role of the endocannabinoid

signaling system in modulating behaviors known to rely at least partially on dopaminergic transmission: locomotion in response to novelty and development of locomotor sensitization to amphetamine. In line with other studies (Klein and Brown, 1969; van Riesen and Leonard, 1990), OBX animals exhibited greater locomotion than sham animals in response to novelty. Sham and OBX animals also responded quite differently to the FAAH inhibitor URB597. In sham animals, URB597 decreased locomotor activity during the preamphetamine interval in a CB₁-dependent manner. By contrast, the FAAH inhibitor did not alter locomotor activity of OBX animals over the same interval. Sham animals also exhibited a time-dependent sensitization to amphetamine that was suppressed by URB597 in a CB₁-dependent manner. In agreement with previous research (Gaddy and Neill, 1976), OBX animals in our study displayed a heightened locomotor response to acute treatment with amphetamine. Our results verify and extend this observation by documenting that, in contrast to shams, further sensitization to amphetamine was absent in OBX animals following repeated amphetamine treatment. Our data suggest that OBX animals are “presensitized” to indirect dopaminergic agonists. Thus, sensitization could not be further enhanced in OBX animals with repeated amphetamine treatment, as suggested previously with repeated cocaine treatment (Chambers et al., 2004). These observations suggest that dopaminergic sensitivity is maximal in OBX animals prior to pharmacological manipulations. This notion is also supported by evidence that dopamine signaling is enhanced in the ventral striatum of OBX rats (Gilad and Reis, 1979; Holmes, 1999; Lingham and Gottesfeld, 1986; Masini et al., 2004). Furthermore, OBX animals were insensitive to the FAAH inhibitor URB597, which suppressed amphetamine sensitization in sham-operated rats. Rimobant alone did not affect sensitization to amphetamine. The rimobant dose employed in the current study (1 mg/kg i.p.) did not alter locomotor activity elicited by exposure to either the open field or to amphetamine. This observation is consistent with previous work documenting that rimobant (1 mg/kg i.p.) blocks pharmacological effects of URB597 without altering basal locomotor activity or inducing anxiogenic-like behavior (Jarbe et al., 2006; Moise et al., 2008; Patel and Hillard, 2006).

In other studies, the CB₁ antagonist/inverse agonist AM251 decreased (Thiemann et al., 2008a) whereas rimobant increased (Masserano et al., 1999; Thiemann et al., 2008b) locomotor responses to amphetamine in otherwise naive animals. In our study, a lower dose (1 mg/kg i.p.) of rimobant was administered than that used in the study by Thiemann et al. (2008b). Thus, this behaviorally inactive dose of rimobant, administered alone, did not affect locomotor activity in our study. Differences in the effects of AM251 and rimobant may be explained by the fact that these drugs are known to have inverse agonist properties and may differentially block CB₁ receptors versus other off-target sites (Pertwee, 2005). However, off-target effects cannot explain the ability of rimobant to block the effects of a FAAH inhibitor in our study.

In sham-operated rats, URB597 decreased the development of amphetamine sensitization in a CB₁-dependent manner. This observation contrasts with the decrease in amphetamine sensitization observed following treatment with AM251 (Thiemann et al., 2008a). Inhibition of anandamide hydrolysis with URB597 (Kathuria et al. 2003) could be expected to exert an effect opposite to that of CB₁ blockade. However, URB597 did not affect development of amphetamine sensitization until day 5 of our paradigm. Subchronic administration of URB597 may increase extracellular anandamide concentrations more effectively in an environment in which the endocannabinoid system is better able to regulate dopaminergic transmission (i.e. elicited in response to novelty or amphetamine exposure). This enhanced modulation may not be apparent until development of amphetamine sensitization is well under way (i.e. approximately day 5 of amphetamine administration in the current study). By contrast, administration of AM251, a CB₁ antagonist/inverse agonist, would exert a more direct, and thus more rapid, effect on CB₁ receptors. Notably, FAAH inhibition had no effect on the development of amphetamine sensitization in OBX animals.

OBX animals exhibited a presensitized locomotor state that is likely to result from an inability of the endocannabinoid system to modulate dopaminergic activity. The olfactory bulbs provide the major cortical input to the olfactory tubercle component of the ventral striatum (Heimer et al., 1995). Olfactory bulbectomy thus removes a major source of glutamatergic afferents in the striatum and possible source of the anandamide synthesizing enzyme NAPE-PLD. Thus, in OBX rats, FAAH inhibition may be unable to reinstate anandamide levels to levels observed in sham-operated rats. Consistent with this hypothesis, OBX animals show altered novelty-induced glutamate release in the striatum relative to sham-operated rats (Ho et al., 2000), providing further evidence for glutamatergic dysregulation in this model. Alternatively, olfactory bulbectomy may disrupt the synthesis of endocannabinoids in the ventral striatum and/or other regions that are deafferented by olfactory bulbectomy. These alterations would be expected to induce lower than optimal levels of endocannabinoids. Finally, limbic structures (e.g. amygdala, piriform and entorhinal cortices) that are deafferented by olfactory bulbectomy (Kelly et al., 1997) may disrupt regulatory input downstream from these structures into the dopaminergic system, including striatal projection neurons. Our data suggest that this disruption may render OBX animals more vulnerable to stimuli, such as novelty and amphetamine, which heighten dopaminergic transmission.

URB597 transiently decreased pre-amphetamine locomotor activity in sham-operated but not OBX animals. This suppression, however, dissipated over time; URB597 no longer suppressed pre-amphetamine locomotor activity by days 4–8 of the sensitization protocol. This interval occurred immediately after injection and may reflect a URB597-mediated acute locomotor effect (i.e. revealed under conditions in which locomotor responses to novelty have undergone habituation following repeated exposure to the open field). As with sensitization, OBX animals were insensitive to this putative locomotor effect of URB597. Interestingly, in sham-operated rats, FAAH inhibition did not influence the development of amphetamine sensitization relative to vehicle-treated controls until later days in the sensitization protocol. Our findings demonstrate that subchronic (8 days) administration of URB597 attenuates the development of locomotor sensitization to amphetamine in sham-operated rats. However, this modulation is notably absent or impaired in OBX rats in which the dopaminergic system is presensitized to stimuli, such as novelty and amphetamine, which heighten dopaminergic transmission. Our data suggest that endocannabinoids and fatty-acid amide hydrolase regulate plasticity in response to stimuli that enhance dopaminergic transmission.

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Figure 1.

Experimental design. All animals underwent the diagrammed procedure daily for 8 days beginning 14 days following OBX or sham surgery. First, animals were placed in the open field arena (30 min pre-injection interval). Then, URB597 (0.3 mg/kg i.p.), URB597 (0.3 mg/kg i.p.) plus rimonabant (1 mg/kg i.p.), rimonabant alone (1 mg/kg i.p.) or vehicle (i.p.) was administered and rats were placed back into the arena (15 min pre-amphetamine interval). Amphetamine (1 mg/kg i.p.) was subsequently administered and rats were placed back into the arena (45 min post-amphetamine interval). Activity levels were monitored for each of the three phases described.

Figure 2.

Novelty-induced locomotor activity in olfactory bulbectomized and sham-operated rats. Olfactory bulbectomy induced a heightened locomotor response to a novel open field relative to sham surgery during the initial open field session (day 1). Data are Mean + SEM; * $P < 0.05$ versus sham (t-test).

Figure 3.

Activity during the pre-amphetamine interval in sham-operated and olfactory bulbectomized rats. In sham animals, URB597 (0.3 mg/kg i.p.) decreased pre-amphetamine locomotor activity (a) on days 1–4 of the sensitization protocol. This effect was blocked by rimonabant (1 mg/kg i.p.). $P < 0.01$ (ANOVA), $*P < 0.05$ for each comparison (Fisher's PLSD post hoc). (b) No changes in pre-amphetamine locomotor activity were observed on days 5–8 of testing. In sham animals, rimonabant (1 mg/kg i.p.) alone did not alter pre-amphetamine locomotor activity relative to vehicle during either the (c) first four or (d) last four days of testing. In OBX rats, URB597 (0.3 mg/kg i.p.) did not affect pre-amphetamine locomotor activity relative to vehicle during either the (e) first four or (f) last four days of testing. Data are Mean + SEM.

Figure 4.

Olfactory bulbectomy alters the development of amphetamine sensitization (a) Olfactory bulbectomy induces a heightened locomotor state in response to initial (day 1) amphetamine (1 mg/kg i.p.) administration $P < 0.01$ (ANOVA) * $P < 0.05$, ** $P < 0.01$ versus sham (t-test) (b) Sham animals develop a typical sensitization to repeated injections of amphetamine, as demonstrated by increased locomotor activity ($P < 0.001$ (Friedman's), + $P < 0.05$ versus day 1 (Dunn's multiple comparisons). By contrast, OBX animals are presensitized to amphetamine. Data are Mean + SEM.

Figure 5.

Effects of cannabinoid pharmacological manipulations on amphetamine sensitization in sham-operated and olfactory bulbectomized rats (a) Locomotor responses to amphetamine (1 mg/kg i.p.) are similar in sham animals that received vehicle (i.p.), URB597 (0.3 mg/kg i.p.), or URB597 (0.3 mg/kg i.p.) coadministered with rimonabant (1 mg/kg i.p.) during the first four days of the sensitization protocol. (b) In sham animals, URB597 (0.3 mg/kg i.p.) attenuated amphetamine sensitization relative to vehicle (i.p.) during the last four days of the sensitization protocol (* $P < 0.05$, ANOVA). This effect was blocked by rimonabant (1 mg/kg i.p.) ($P < 0.05$ for each comparison, Fisher's PLSD post hoc). Rimonabant (1 mg/kg i.p.) did not affect the development of amphetamine sensitization during either the (c) first four or (d) the last four

days of testing. In OBX animals, URB597 did not alter the development of amphetamine sensitization during either the (e) first four or (f) last four days of testing. Data are Mean + SEM.