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Inhibition of fatty acid amide hydrolase modulates anxiety-like behavior in PCP-treated rats

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ABSTRACT

Sub-chronic administration of PCP produces a social interaction deficit that is reversed by URB597, an inhibitor of the catabolic enzyme of the endocannabinoid anandamide. Since increased anxiety may contribute to social withdrawal and URB597 has been shown to have an anxiolytic action, we studied whether this drug affected saline- and PCP-treated rats' performance in the Elevated-Plus-Maze task, which has been used to assess anxiety-like effects. Sub-chronic PCP produced a CB_1-dependent decrease in anxiety-like behavior that was reversed by URB597 in a CB_1-independent fashion, as it was not blocked by the CB_1 antagonist AM251. These findings suggest that PCP-treated rats have altered endocannabinoid transmission and that anxiety does not contribute to the PCP-induced social withdrawal.

1. Introduction

In humans, consumption of the N-methyl-D-aspartate (NMDA) glutamate receptor antagonist, phencyclidine (PCP) evokes not only the positive and negative symptoms of schizophrenia, but also the characteristic cognitive deficits observed in the disease (Murray, 2002). The similarity between the behavioral effects of PCP and schizophrenia symptomatology has led to the development of pharmacological models based on repeated PCP administration (Enomoto et al., 2007). In rats, withdrawal from sub-chronic PCP reduces social interaction (Lee et al., 2005; Seillier and Giuffrida, 2009), a behavioral phenotype reminiscent of negative symptoms.

The FAAH inhibitor URB597, a drug that elevates the endocannabinoid anandamide by blocking its degradation (Fegley et al., 2005), has been shown to reverse social withdrawal in PCP-treated rats (Seillier et al., 2010), suggesting an antipsychotic activity. There is emerging evidence, however, for a role of the endocannabinoid system in the control of anxiety (Lafenêtre et al., 2007), which may represent a confounder for the interpretation of these results. Given the contribution of anxiety to social interaction (File and Seth, 2003) and the ability of URB597 to reduce anxiety-related behaviors (Moreira et al., 2008; Moise et al., 2008; Scherma et al., 2008; Haller et al., 2009), we assessed whether URB597 affected the performance of saline- and PCP-treated rats in the Elevated-Plus-Maze (EPM), a well-established behavioral test of anxiety (Lapiz-Bluhm et al., 2008), under the same experimental conditions used in our social interaction paradigm (Seillier et al., 2010).

2. Material and methods

2.1. Animals and drug treatment

Male Wistar rats (200–225 g; Charles River Laboratories, USA) were housed at 22 ± 1 °C under a 12 h light/dark cycle with food and water available ad libitum. Animals were habituated to housing conditions for 1 week and behavioral testing was carried out during the light cycle. Two experimental groups were treated sub-chronically with either saline (1 ml/kg, i.p.) or PCP (5 mg/kg, i.p.; Sigma/RBI, USA) b.i.d. for 7 days and tested 5 days after the last drug injection (Seillier et al., 2010). Under this regimen, we did not observe any sign of drug abstinence (e.g. piloerection, weight loss, hyper-responsiveness, bruxism). All experiments were carried out according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the IACUC of the University of Texas Health Science Center at San Antonio.

2.2. Apparatus and procedures

Anxiety-like behavior was assessed using the EPM test five days after the last drug (PCP or saline) injection; no PCP was on board or given on the day of test. The EPM (Columbus Instruments, USA) was made of stainless steel and consisted of four arms (10 cm wide × 50 cm long) fixed to a central platform (10 × 10 cm): the two closed arms had 40 cm high walls, whereas the two open arms had a

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0.5 cm raised edge. The EPM (100 cm from the floor) was located in a dimly lit room (9 lux at the EPM central platform) as in our social interaction paradigm (Seillier et al., 2010). Diazepam (1 mg/kg, i.p.) was used to assess the effects of a standard anxiolytic drug in our low-light conditions. Rats were acutely injected with either vehicle (Tween-80:PEG:saline, 5:5:90, 1 ml/kg, i.p.) or URB597 (0.1, 0.3 or 1.0 mg/kg, i.p.; synthesized by the Southwest Research Institute, San Antonio) 1 h before the test, individually placed in the center of the EPM facing an open arm and allowed to explore the maze for 5 min. To investigate the pharmacological mechanisms underlying PCP and/or URB597 effects on anxiety, rats were pre-treated with the selective CB_1 antagonist AM251 (1 mg/kg, i.p.; Ascent) or its vehicle (Tween-80:PEG:saline, 10:10:80, 1 ml/kg, i.p.) immediately before URB597 (0.3 mg/kg, i.p.). The maze was wiped clean with 10% ethanol between each test. Data were collected by an experimenter blind to the study as total number of entries ($TE = E_{open} + E_{closed} + E_{center}$) and time spent in each zone (the center zone was coded as neither open nor closed arms). An entry was defined as a rat entering the arm with all four paws. Levels of anxiety were assessed as percent time spent in the open arms [$\%T_{open} = T_{open}/(T_{open} + T_{closed})$] and percent of open arm entries [$\%E_{open} = E_{open}/(E_{open} + E_{closed})$].

2.3. Statistical analysis

Statistical analysis was carried out by two-way ANOVA with Treatment (saline, PCP) and URB597 Dose (0.0–1.0 mg/kg) as between-subject factors or by three-way ANOVA with Treatment, Drug (vehicle, URB597) and Antagonist (vehicle, AM251) as between-subject factors, and followed by Newman-Keuls *post-hoc* comparisons.

3. Results

PCP-treated rats showed significantly higher $\%T_{open}$ (Fig. 1A; $p<0.01$) and $\%E_{open}$ (Fig. 1B; $p<0.05$) compared to saline-treated controls, which resembled the anxiolytic response induced by diazepam [$\%T_{open}$: $F(1,11)=19.17$, $p<0.01$;$\%E_{open}$: $F(1,11)=17.40$, $p<0.01$; TE: $F(1,12)=3.10$, NS]. In saline-treated rats, URB597 produced a slight, but not significant, increase of $\%T_{open}$ (Fig. 1A) and $\%E_{open}$ (Fig. 1B). On the other hand, URB597 reversed the PCP-induced increase in $\%T_{open}$ and $\%E_{open}$ at all doses with a maximum effect at 0.3 mg/kg (Fig. 1A; $p<0.01$ and Fig. 1B; $p<0.01$). Statistical analysis showed a significant interaction between Treatment and URB597 Dose for $\%T_{open}$ [$F(1,3)=7.08$, $p<0.001$] and $\%E_{open}$ [$F(1,3)=6.53$, $p<0.001$], but not for TE [$F(1,3)=2.08$, NS; Fig. 1C].

The CB_1 antagonist AM251, which alone had no effect on EPM behaviors, reversed PCP-induced increases in $\%T_{open}$ (Fig. 2A; $p<0.001$) and $\%E_{open}$ (Fig. 2B; $p<0.01$). The slight, but not significant, anxiolytic-like action of URB597 was not observed in saline-treated rats after AM251 administration. However, AM251 did not block URB597 anxiogenic effect in PCP-treated rats. Statistical analysis showed a significant interaction among all factors (Treatment, Drug and Antagonist) for $\%T_{open}$ [$F(1,1)=8.34$, $p<0.01$] and a similar trend for $\%E_{open}$ [$F(1,1)=3.72$, $p=0.059$], but not for TE [$F(1,1)=0.37$, NS; Fig. 2C].

4. Discussion

Our results clearly show that PCP-treated rats have reduced anxiety-like behavior in the EPM and that this behavior is reversed by pharmacological blockade of CB_1 receptors, as well as by enhancement of anandamide tone via systemic administration of URB597. In contrast to previous reports which show a CB_1-dependent anxiolytic action of URB597 in normal animals (Moreira et al., 2008; Moise et al., 2008; Haller et al., 2009), this drug has a CB_1-independent anxiogenic effect in PCP-treated rats.

To date, no changes in anxiety levels, as assessed by the EPM, open field and light-dark tests, have been shown in the sub-chronic PCP rat model of schizophrenia (Lee et al., 2005; Schwabe et al., 2006; McLean et al., 2010), with the exception of one report from Audet et al. (2007), showing increased anxiety in the light-dark test and during exposure to a cat odor. Surprisingly, our data suggest that sub-chronic PCP reduces anxiety behavior in the EPM test to a similar extent as a dose of 1 mg/kg of diazepam, a standard anxiolytic drug. The discrepancies between our data and previous observations may be attributed to the different strains of rats, dose of PCP and/or the experimental tests and parameters used. Specifically, our rats were tested under very low illumination, as reported in our social interaction study (Seillier et al., 2010). Although some studies show that rats' behavior in the EPM is insensitive to light conditions (Becker and Grecksch, 1996), others indicate that light can be aversive in this test (Griebel et al., 1993; Bertoglio and Carobrez, 2002). Interestingly, Handley et al. (1993) showed that the serotonin 5-HT_{1A} receptor agonist 8-OH-DPAT is anxiolytic under high illumination, but anxiogenic under dim light. Thus, a similar scenario may account for the inconsistencies between our results and previously published data.

Although experimental evidence indicates that URB597 has CB_1-dependent anxiolytic effects in mice (Moreira et al., 2008) and hamsters (Moise et al., 2008), the reports in rats have been contradictory (Naderi et al., 2008; Scherma et al., 2008), possibly because the anxiolytic properties of URB597 become evident only under aversive conditions (Naidu et al., 2007; Haller et al., 2009). In agreement with these studies, we did not observe any significant anxiolytic effect of URB597 in saline-treated rats under dim light. As previously reported (Haller et al., 2007, 2009), AM251 did not affect

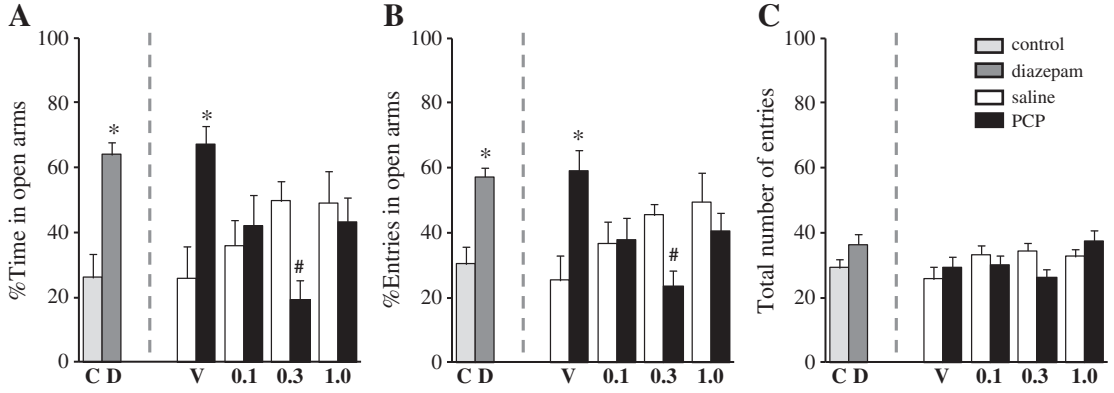

Fig. 1. Effects of URB597 (0.1–1.0 mg/kg) in saline- and PCP- treated rats on (A) percent time spent in the open arms ($\%T_{open}$), (B) percent of open arms entries ($\%E_{open}$) and (C) total number of entries (TE) in the EPM. For comparison, diazepam effects (1 mg/kg) in an independent group of control rats are also reported on the right panel of each graph. Values are expressed as mean ± S.E.M. ($n=8$ per group). * $p<0.05$ compared to saline-treated rats; # $p<0.05$ compared to corresponding vehicle (V) controls.

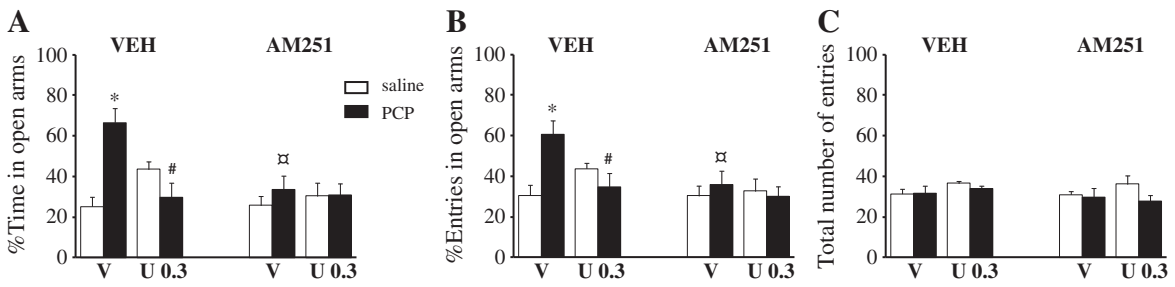

Fig. 2. Effects of AM251 (1.0 mg/kg) on PCP- and URB597- (0.3 mg/kg) induced changes in (A) percent time spent in the open arms ($\%T_{open}$), (B) percent of open arms entries ($\%E_{open}$) and (C) total number of entries (TE) in the EPM. Values are expressed as mean ± S.E.M. (n = 8 per group). * $p<0.05$ compared to corresponding saline-treated rats; # $p<0.05$ compared to URB597 vehicle (V) controls; ¤ $p<0.05$ compared to AM251 vehicle (VEH) controls.

anxiety in saline-treated rats, but it did reverse the decreased anxiety in PCP-treated rats, suggesting that increased activity at CB_1 receptors contributes to the anxiolytic-like behavior in this experimental group. In support of this hypothesis, PCP-treated rats showed enhanced anandamide levels in the nucleus accumbens (NAc) and a similar trend in the medial prefrontal cortex (mPFC) (Seillier et al., 2010), two brain areas involved in anxiety regulation (Martinez et al., 2002; Shah and Treit, 2003), as well as increased 2-arachidonylglycerol levels in the mPFC (Vigano et al., 2009; Guidali et al., 2011). Interestingly, decreased anxiety-like behavior in dopamine D_3 receptor knockout mice has been associated with higher levels of the endocannabinoid 2-arachidonylglycerol in the NAc, and reversed by AM251 (Micale et al., 2009a). Furthermore, Δ^9-tetrahydrocannabinol microinjection in the rat mPFC has been shown to induce a CB_1-dependent anxiolytic response (Rubino et al., 2008a).

Given the CB_1-dependent anxiolytic-like effect of PCP treatment and the anxiolytic properties of URB597 reported in the literature (Moreira et al., 2008; Moise et al., 2008; Scherma et al., 2008; Haller et al., 2009), the observation that URB597 produced an anxiogenic effect in PCP-treated rats was puzzling. However, Scherma et al. (2008) showed that, in normal rats, anandamide or URB597 are anxiolytic when administered alone, but produce an anxiogenic effect when combined, suggesting that marked increases of anandamide levels may lead to anxiogenic responses. Indeed, Rubino et al. (2008b) found a CB_1-dependent anxiolytic effect of URB597 infusion in the mPFC at low doses, but a CB_1-independent and TRPV1-mediated anxiogenic effect at higher doses. Therefore, as PCP-treated rats have already significantly elevated anandamide levels compared to normal controls (Seillier et al., 2010), administration of URB597 in PCP-treated rats might shift anandamide effects from anxiolytic (CB_1-dependent) to anxiogenic (CB_1-independent, likely TRPV1-dependent) by further enhancing anandamide levels. This hypothesis is in agreement with recent data showing opposite functional roles for CB_1 and TRPV1 receptors (Morgese et al., 2007; Rubino et al., 2008b; Micale et al., 2009b), and a well-established participation of TRPV1 in anxiety (Marsch et al., 2007; Santos et al., 2008; Aguiar et al., 2009; Terzian et al., 2009). Noteworthily, we previously showed that URB597 was equally effective at enhancing anandamide levels in saline- and PCP-treated rats (Seillier et al., 2010), suggesting that PCP does not alter URB597 inhibitory activity at FAAH.

In conclusion, our data indicate that sub-chronic PCP produced a CB_1-dependent decrease in anxiety-like behavior that was reversed by URB597 in a CB_1-independent fashion. In addition, the social withdrawal observed in PCP-treated rats (Seillier et al., 2010) cannot be attributed to increased anxiety, confirming previously published data showing insensitivity of this behavioral deficit to anxiolytic drugs (Sams-Dodd, 1998; Snigdha and Neill, 2008). The CB_1-dependent PCP-induced reduction of anxiety-like behavior may result from anandamide elevation in brain areas involved in anxiety-related regulation. However, further elevation of anandamide tone by URB597 may lead to the recruitment of non-CB_1 targets, such as TRPV1 receptors, and, therefore, produce an anxiogenic effect.

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