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Chronic FAAH inhibition during nicotine abstinence alters habenular CB1 receptor activity and precipitates depressive-like behaviors

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ABSTRACT

The role of the endocannabinoid system in nicotine addiction is being increasingly acknowledged. Acute inhibition of anandamide (AEA) degradation efficiently reduces nicotine withdrawal-induced affective symptoms in rats and fatty acid amide hydrolase (FAAH), the degradation enzyme of AEA, has been proposed as a possible treatment against nicotine addiction. However, it is unclear whether chronic inhibition of AEA during nicotine abstinence will have beneficial or deleterious affective side-effects. Using a rat model of nicotine addiction, we found that, during abstinence, rats injected daily with a FAAH inhibitor (URB597) developed a depressive-like phenotype. Our results show that in the nicotine abstinent rats, URB597 induced low saccharin consumption, persistent immobility in the forced swim test and increased corticosterone levels in response to stress. In addition, URB597decreased CB1 receptor binding and activity in the habenula, a key structure in the control of nicotine-related emotional states. In contrast, non-treated abstinent rats showed increased CB1 receptor activity and behaviors comparable to controls. No FAAH inhibition-induced alterations were observed in animals that had a previous history of saline self-administration. Taken together, our results suggest that chronic FAAH inhibition prevents the homeostatic adaptations of habenular CB1 receptor function that are necessary for the recovery from nicotine dependence.

1. Introduction

Smoking cessation in humans is often associated with negative somatic and affective symptoms such as irritability, anxiety and depressed mood, which contribute to the high probability of smoking relapse. The discovery of the endocannabinoid system has provided potential targets for the treatment of mood as well as addictive-related disorders. Rimonabant, a cannabinoid CB1 receptor inverse agonist, increases the rate of smoking abstinence, however its therapeutic potential is limited by significant side-effects associated with its repeated use (Cahill and Ussher, 2007). Importantly, these results highlight that the failure of candidate medications to translate from bench to bedside is often due to their detrimental long term effects.

We and others have successfully assessed the beneficial acute effects of several endocannabinoid compounds, including CB1 agonists, antagonists and fatty acid amide hydrolase (FAAH) inhibitors on nicotine-taking and seeking behaviors (Le Foll et al., 2014; Muldoon et al., 2013; Simonnet et al., 2013). However, we still lack preclinical examination of the consequences of chronic treatments with the same compounds in nicotine abstinent subjects and the development of potential side-effects.

Over the last few years, an important body of evidence has emerged implicating the endocannabinoid system in the regulation of nicotine reward and withdrawal. Genetic CB1 deletion or CB1 receptor antagonists reduce nicotine-induced hyperactivity (Castane et al., 2002), conditioned place preference (Forget et al., 2005), nicotine intravenous self-administration (Simonnet et al., 2013) and cue-induced reinstatement of nicotine seeking behavior (Cohen et al., 2005). Moreover, the development of the CB2 receptor pharmacology has indicated that acute blockade of the CB2 receptors can decrease nicotine reward-related behaviors (Navarrete et al., 2013); but see (Gamaleddin et al., 2012).

Surprisingly, there are similarities between the effect of acute elevation of anandamide (AEA), and thus CB1 receptor signaling, and those of CB1 receptor antagonists. Drugs that elevate AEA such

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as AEA uptake inhibitors (i. e. VDM 11 and AM404) decrease nicotine seeking (Gamaleddin et al., 2011, 2013) and FAAH inhibitors have recently been suggested as a new therapy for various psychiatric disorders, including the treatment of nicotine addiction (Forget et al., 2016). Acute FAAH inhibition blocks both the development and the reinstatement of nicotine-induced conditioned place preference, reducing acquisition of nicotine intravenous self-administration and nicotine-seeking behavior in rats (Forget et al., 2009; Scherma et al., 2008). Furthermore, FAAH inhibition significantly decreases nicotine-induced elevations in dopamine levels in the nucleus accumbens shell (Scherma et al., 2008). Acute FAAH inhibition does not prevent the expression of nicotine withdrawal-precipitated somatic symptoms, but it does reduce the negative affective symptoms observed upon acute nicotine cessation (Cippitelli et al., 2011), suggesting that it might ameliorate the negative affective state of prolonged nicotine abstinence and vulnerability to relapse. However, chronic inhibition of FAAH has led to conflicting data. Indeed, genetic FAAH deletion enhances nicotine withdrawal-induced affective and somatic signs in mice (Merritt et al., 2008). It should be noted that compensatory changes may have occurred in the FAAH KO mice as a result of the genetic deletion. Nevertheless, the therapeutic potential of chronic FAAH inhibition clearly remains an open question.

Here, we examined whether chronic administration of a FAAH inhibitor (URB597) during nicotine abstinence promotes negative symptoms. After a two-month nicotine self-administration period, abstinent rats were injected daily with URB597 (0.3 mg/kg) and different behavioral tests were performed to assess emotional states. In addition, we analyzed CB1 receptor binding and functionality in several brain areas, including the habenula (Soria-Gómez et al., 2015) based on its implication in both nicotine addiction and depressive symptoms (Baldwin et al., 2011; Fowler and Kenny, 2014). Control animals were run in parallel for saline self-administration and received the same URB597 treatment and behavioral assessment.

2. Materials and methods

2.1. Animals

Thirty-three male Sprague Dawley rats (Charles River, 175–200 g) were used in this experiment. Rats were housed 3 per cage and maintained in a room at 20–22 °C with lights off from 8h to 20 h. Thus, all experiments were conducted during the active phase. In addition, rats were given a diet of 20 g/day lab chow which is sufficient to maintain normal body weight and growth during the whole experiment. Water was available *ad libitum* and food was given at the end of each day. Experiments started after 1-week of acclimatization in the animal room.

2.2. Drugs

Nicotine hydrogen tartrate (Sigma-Aldrich, France) was dissolved in sterile physiological saline (0.9%), and concentrations are expressed in nicotine base (1 mg nicotine base = 2.85 mg nicotine tartrate). The nicotine solution for subcutaneous injection was pH adjusted to 7.4. URB597 (Sigma-Aldrich, France) was dissolved in a vehicle made of ethanol-Cremophor-saline (1:1:18) (Cremophor, Sigma-Aldrich, France).

2.3. Intravenous nicotine self-administration

2.3.1. Surgery

Rats were anesthetized using isoflurane and equipped with chronic indwelling SILASTIC catheters in the right jugular vein, as described previously (Reisiger et al., 2014). During the first week after surgery the catheters were flushed daily with 0.2 ml of ampicillin solution (0.1 g/ml; Coophavet) containing heparin (300 IU/ml). When necessary, catheter patency was verified with an infusion of the short-acting barbiturate Hypnomidate® (2 mg/ml, Janssen-Cilag).

2.3.2. Apparatus

Self-administration was performed using operant chambers (30 × 40 × 37 cm, Imetronic) equipped with two nose-poke devices ("active" and "inactive"). External pumps were fitted with syringes that were connected with Tygon tubing to a fluid swivel and weighted pulley system inside the chamber. The swivel was connected to a spring connector and attached to the back-mount of the animal. Illumination of a dim house-light signaled the start of each 2 h test session. Responses in the active hole resulted in a 0.1 ml infusion of solution across 4 s. Each infusion was accompanied by a compound visual stimulus consisting of a 3 s cue light above the active operandum and a time-out period (20 s). Responses on the active hole during the time out or on the inactive operandum at any time were recorded, but were of no consequence. A white noise was continually present during the experiment.

2.3.3. Nicotine i.v. self-administration acquisition and maintenance

Self-administration session began with a single non-contingent infusion of nicotine (NIC, n = 20) at a dose of 60 μg/kg/0.1 ml and lasted for 2 h. Infusions were earned on a fixed ratio (FR) schedule of reinforcement (10 days FR1, 2 days FR2, stabilizing for 47 days on FR5) [see timeline Fig. 1]. A control group was run in parallel (SAL, n = 13). SAL-treated animals received saline solution on the same schedule of reinforcement (NaCl 0.9%, 0.1 ml/infusion).

2.4. Chronic treatment with URB597

On the 8th day after nicotine self-administration cessation [see timeline Fig. 1], rats received their first daily injection (at 6pm) of either vehicle (1 ml/kg) or URB597 (0.3 mg/kg/ml i.p.). Animals were then injected daily for 6 weeks until the end of the experiment. Saline exposed rats were renamed SAL-V (n = 7) and SAL-URB (n = 6) and nicotine exposed rats were renamed NIC-V (n = 10) and NIC-URB (n = 10). All behavioral experiments started at 9 a.m. (or 8 a.m. for saccharin consumption test), which allowed testing under relevant time conditions of FAAH inhibition (Fegley et al., 2005).

2.5. Saccharin consumption test

Cessation of nicotine administration results in a withdrawal syndrome characterized by anhedonia (i.e., an inability to experience pleasure). Anhedonia was evaluated in individually housed rats using the two-bottle choice test. At 8 a.m. on the day of testing, animals were transferred to the experimental room in individual cages with no access to food or water for the duration of the test. They were given one bottle that contained saccharin solution (0.13%) and another that contained drinking water. The animals had access to both solutions for 1 h without prior water deprivation. The positions of the two drinking bottles were randomized. At the end of the test, rats were returned to their collective home-cages in the colony room. Relative saccharin intake (ml/kg) was measured during nicotine withdrawal (at day 7) and during protracted abstinence (at day 41) [timeline Fig. 1 (1) and (4)].

2.6. Activity recording

Locomotor activity was measured in activity cages (35 × 25 × 25 cm) with wire mesh floors and 10 mm Plexiglas