Neuropharmacology 63 (2012) 1335–1345

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Nicotine-induced anxiety-like behavior in a rat model of the novelty-seeking phenotype is associated with long-lasting neuropeptidergic and neuroplastic adaptations in the amygdala: Effects of the cannabinoid receptor 1 antagonist AM251

Cigdem Aydin, Ozge Oztan, Ceylan Isgor*

Charles E. Schmidt College of Medicine, Department of Biomedical Science, Florida Atlantic University, 777 Glades Road, Boca Raton, FL 33431, USA

ARTICLE INFO

Article history:
Received 6 December 2011
Received in revised form
15 August 2012
Accepted 20 August 2012

Keywords:
Behavioral sensitization
Social anxiety
Cannabinoid receptor 1
Nicotine abstinence
Amygdala
Neuroplasticity

ABSTRACT

A rat model of the novelty-seeking phenotype predicts vulnerability to the expression of behavioral sensitization to nicotine, where locomotor reactivity to novelty is used to screen experimentally-naïve rats for high (HR) versus low (LR) responders. The present study examines the long-term neuropeptidergic and neuroplastic adaptations associated with the expression of locomotor sensitization to a low dose nicotine challenge and social anxiety-like behavior following chronic intermittent nicotine exposure during adolescence in the LRHR phenotype. Our data show that the expression of behavioral sensitization to nicotine and abstinence-related anxiety are detected in nicotine pre-exposed HRs even across a long (3 wks) abstinence. Moreover, these behavioral effects of nicotine are accompanied by a persistent imbalance between neuropeptide Y and corticotrophin releasing factor systems, and a persistent increase in brain-derived neurotrophic factor (BDNF) and spinophilin mRNA levels in the amygdala. Furthermore, treatment with the cannabinoid receptor 1 antagonist, AM251 (5 mg/kg) during a short (1 wk) abstinence is ineffective in reversing nicotine-induced anxiety, fluctuations in BDNF and spinophilin mRNAs, and the neuropeptidergic dysregulations in the amygdala; although this treatment is effective in reversing the expression of locomotor sensitization to challenge nicotine even after a long abstinence. Interestingly, the identical AM251 treatment administered during the late phase of a long abstinence further augments anxiety and associated changes in BDNF and spinophilin mRNA in the basolateral nucleus of the amygdala in nicotine pre-exposed HRs. These findings implicate long-lasting neuropeptidergic and neuroplastic changes in the amygdala in vulnerability to the behavioral effects of nicotine in the novelty-seeking phenotype.

Published by Elsevier Ltd.

1. Introduction

The novelty-seeking phenotype has been shown to predict individual differences in vulnerability to psychostimulant sensitization in rodents (Hooks et al., 1991; Dietz et al., 2005; Cain et al., 2009). This phenotype is identified by the level of locomotor reactivity to a novel environment in an experimentally-naïve outbred population of rats. Specifically, some rats exhibit high locomotor response to novelty and are identified as high responders, HRs; whereas some display low locomotor response to novelty and are identified as low responders, LRs. Our laboratory previously reported expression of locomotor sensitization to a low dose nicotine challenge following a chronic intermittent nicotine exposure during the peripubertal–juvenile period and 1 wk of abstinence in the HR, but not LR rats that is reversed by a cannabinoid receptor 1 (CB1R) antagonist, AM251, administration (Bhatti et al., 2009); implicating CB1R signaling in sensitizing effects of nicotine.

Clinical studies demonstrated effectiveness of a commercially available CB1R antagonist, rimonabant, in increasing prolonged abstinence rates compared to placebo (Le Foll et al., 2008). However, due to its neuropsychiatric side effects, such as anxiety, rimonabant has been disapproved by the FDA (US Food and Drug Administration, 2007; Bermudez-Silva et al., 2010; Cahill and

Abbreviations: BLA, basolateral nucleus of the amygdala; BDNF, brain-derived neurotrophic factor; CB1R, cannabinoid receptor 1; CeA, central nucleus of the amygdala; CRF, corticotrophin releasing factor; EPM, elevated plus maze; HR, high responder; LR, low responder; MeA, medial nucleus of the amygdala; NPY, neuropeptide Y; SI, social interaction.

* Corresponding author. Tel.: +1 561 297 0712; fax: +1 561 297 2519.
E-mail addresses: caydin@fau.edu (C. Aydin), cisgor@fau.edu (C. Isgor).

0028-3908/$ – see front matter Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.neuropharm.2012.08.016

Ussher, 2011). In confirmation of the clinical findings, studies on rodents showed that rimonabant reduces nicotine self-administration (Cohen et al., 2002), as well as the expression of locomotor sensitization to low dose nicotine challenge (Bhatti et al., 2009), but also increases anxiogenic-like responses to nicotine (Balerio et al., 2006). These data suggest involvement of CB1R signaling in nicotine-induced anxiogenesis. Indeed, we have recently reported a decrease in CB1R mRNA levels in the amygdala along with emergence of social anxiety-like behavior in HRs following repeated nicotine exposure in the peripubertal–juvenile period (Aydin et al., 2012), suggesting that a deficit in CB1R function in the amygdala may be a substrate for nicotine-induced anxiety-like behavior.

Previous reports from our laboratory showed that a behaviorally-sensitizing nicotine regimen results in increased abstinence-related social anxiety-like behavior, which is accompanied by a deficit in neuropeptide Y (NPY) mRNA levels in the medial nucleus of amygdala (MeA) together with increased corticotrophin releasing factor (CRF) mRNA levels in the central nucleus of amygdala (CeA; Aydin et al., 2011a, 2011b). Moreover, our data also showed that systemic antagonization of the presynaptic Y2 receptors during 1 wk of abstinence by way of peripheral injections of a novel, brain penetrant Y2 receptor antagonist, JNJ-31020028 reestablishes the critical homeostatic balance between the amygdalar NPY and CRF systems and reverses the expression of behavioral sensitization to a low dose nicotine challenge and associated social anxiety-like behavior in the nicotine vulnerable HR rats (Aydin et al., 2011b). These findings suggest a relationship between the emergence of anxiety-like behavior and the neuropeptidergic changes within the amygdala in behavioral sensitization to nicotine in the novelty-seeking phenotype. However, the role of CB1R signaling in the nicotine-induced negative affective state and the neuropeptidergic adaptations in the amygdala remain to be elucidated.

In addition to NPY and CRF, amygdalar BDNF also mediates anxiety-like behavior. Data from BDNF overexpressing mice show that these animals have upregulated BDNF levels in the basolateral nucleus of amygdala (BLA), and display increased anxiety-like behavior in the open field and elevated plus maze (EPM) tests, compared to controls (Govindarajan et al., 2006), suggesting a modulatory role for amygdalar BDNF in anxiety-like behavior. Moreover, BDNF promotes dendritic and spine growth (Ji et al., 2005) and the observed increase in anxiety-like behavior in the BDNF overexpressing mice strongly correlates with increased spine density in the BLA, suggesting that increased spinogenesis in the amygdala may be a cellular substrate for enhanced anxiety (Govindarajan et al., 2006). Indeed, such correlation between increased anxiety and BLA spinogenesis has also been reported following chronic stress (Bennur et al., 2007). Additionally, an upregulation in levels of spinophilin in the amygdala is reported following a behaviorally-sensitizing amphetamine regimen and 4 wks of abstinence (Boikess and Marshall, 2008), suggesting a long-lasting dendritic plasticity in the amygdala with psychostimulant administration. However, the role of BDNF and subsequent neuroplasticity in the amygdala has not received attention in a nicotine-induced anxiety paradigm.

In the present study, we have investigated if there are long-lasting behavioral, neuropeptidergic and neuroplastic adaptations following repeated nicotine exposure during the peripubertal–juvenile period in the LRHR rats. In doing so, we assessed whether nicotine exposure during the peripubertal juvenile period can induce long-lasting changes in the expression of behavioral sensitization to nicotine, social anxiety-like behavior, dysregulations in the amygdalar NPY and CRF as well as fluctuations in the molecular markers of neuroplasticity (i.e., BDNF and spinophilin mRNAs) in the amygdala in LRHR rats. To determine long-lasting nature of these parameters, we employed a short (i.e., 1 wk) and a long (i.e., 3 wks) abstinence spanning into young adulthood following nicotine training. We also assessed if AM251 administered during the short or late phase (i.e., last week) of the long abstinence sufficiently reverses the nicotine-induced behavioral and neural adaptations in the LRHR rats.

2. Materials and methods

2.1. Drugs

Nicotine hydrogen tartrate was obtained from a commercial supplier (Sigma), dissolved in 0.9% NaCl and the pH was adjusted to 7.4. AM251 was obtained from Tocris Bioscience and dissolved in a vehicle solution consisting of Tween 80, DMSO and 0.9% NaCl with the proportion of 1:2:7. This specific proportion has been used to formulate the vehicle solution to dissolve AM251 in a number of studies in the literature (Hoffman et al., 2007; Rubino et al., 2008). The doses for nicotine and AM251 were chosen based on effective doses used in the literature in several reports by others (Miller et al., 2001; Suto et al., 2001; Le Foll and Goldberg, 2004), and by us (Bhatti et al., 2007, 2009; Aydin et al., 2011a, 2011b, 2012).

2.2. Animal housing and the LRHR phenotype screening

Animals were treated in accordance with the National Institute of Health guidelines on laboratory animal use and care. All efforts were made to minimize animal suffering and to reduce the number of animals used. A grand total of 216 male Sprague-Dawley rats (Charles River, Wilmington, MA) arrived at weaning (postnatal day, PN 22), were housed 3 per cage in $43 \times 21.5 \times 25$ cm^3 Plexiglas cages and were kept on a 12 h light/dark cycle (lights on at 7:00 A.M.). Food and water were available *ad libitum*. Animals were allowed to habituate to the housing conditions and were handled daily for 2 days. On PN 25, animals were screened for locomotor reactivity to the mild stress of a novel environment for 1 h using commercially available locomotion chambers (San Diego Instruments, San Diego, CA). Briefly, locomotor reactivity to novelty was tested in $43 \times 43 \times 24.5$-cm^3 (high) clear Plexiglas cages with stainless steel grid flooring. Activity was monitored by means of photocells (a total of $X = 16$ by $Y = 16$ photocells) 2.5 cm above the grid floor and equally spaced along the sides of the box. Horizontal locomotion was monitored by this lower bank of photocells. Each locomotor count recorded a minimum of 3-cm traversing of the cage. Additional photocells were located 11.5 cm above the grid floor and 9 cm above the lower bank of photocells. Rearing (i.e., locomotion in the Z plane) was monitored by this upper bank of photocells. At the end of the screening session, total locomotor activity (i.e., X, Y, and Z locomotion) was pooled and the rats were ranked as HRs (i.e., rats that exhibited locomotor scores in the highest third of the sample; $n = 72$) or LRs (i.e., rats that exhibited locomotor scores in the lowest third of the sample $n = 72$). The intermediary responders were used as residents rats in the social interaction (SI) test described below.

2.3. Behavioral sensitization to nicotine and abstinence-related anxiety-like behavior

The behavioral sensitization to nicotine procedure is similar to Table 1 without the challenge phase. Male Sprague-Dawley rats arrived on PN 22 and were phenotype screened on PN 25. Following phenotype screening, the LRHR animals were assigned to saline (1 ml/kg; s.c.) or nicotine (0.35 mg/kg; s.c.) injection groups. On injection days, rats were given 1 h to habituate to the locomotor chambers before they received an injection of the assigned drug. Their locomotor response was recorded for 90 min. This procedure was repeated four times at a 3-d interval. Following the fourth injection, half of the animals in each group underwent 1 wk of abstinence, while the remaining half underwent 3 wks of abstinence ($n = 5$–6/group). In the present study, the term "abstinence" is used to refer to the period starting after the 4th nicotine injection and lasting until the day of low dose nicotine challenge, during which nicotine is withdrawn by the experimenter. At the end of the abstinence periods, all animals were tested on the SI test for assessing anxiety-like behavioral profile as described below, in the absence of nicotine.

2.4. AM251 treatment and low dose nicotine challenge-induced anxiety

The behavioral sensitization to nicotine procedure and the experimental groups used in the present study are outlined in Tables 1 and 2, respectively. Male Sprague-Dawley rats arrived on PN 22 and were allowed to rest until PN 25, at which time they were phenotype screened. Following phenotype screening, the LRHR animals were assigned to saline (1 ml/kg; s.c.) or nicotine (0.35 mg/kg; s.c.) injection groups. On injection days, rats were given 1 h to habituate to the locomotor chambers before they received an injection of the assigned drug. Their locomotor response was recorded for 90 min. This procedure was repeated four times at a 3-d interval.