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Research report

Effects of a selective Y2R antagonist, JNJ-31020028, on nicotine abstinence-related social anxiety-like behavior, neuropeptide Y and corticotropin releasing factor mRNA levels in the novelty-seeking phenotype

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ABSTRACT

An outbred rat model of novelty-seeking phenotype has predictive value for the expression of locomotor sensitization to nicotine. When experimentally naïve rats are exposed to a novel environment, some display high rates of locomotor reactivity (HRs, scores ranking at top 1/3rd of the population), whereas some display low rates (LRs, scores ranking at bottom 1/3rd of the population). Basally, HRs display lower anxiety-like behavior compared to LRs along with higher neuropeptide Y (NPY) mRNA in the amygdala and the hippocampus. Following an intermittent behavioral sensitization to nicotine regimen and 1 wk of abstinence, HRs show increased social anxiety-like behavior in the social interaction test and robust expression of locomotor sensitization to a low dose nicotine challenge. These effects are accompanied by a deficit in NPY mRNA levels in the medial nucleus of the amygdala and the CA3 field of the hippocampus, and increases in Y2R mRNA levels in the CA3 field and corticotropin releasing factor (CRF) mRNA levels in the central nucleus of the amygdala. Systemic and daily injections of a Y2R antagonist, JNJ-31020028, during abstinence fully reverse nicotine-induced social anxiety-like behavior, the expression of locomotor sensitization to nicotine challenge, the deficit in the NPY mRNA levels in the amygdala and the hippocampus, as well as result an increase in Y2R mRNA levels in the hippocampus and the CRF mRNA levels in the amygdala in HRs. These findings implicate central Y2R in neuropeptidergic regulation of social anxiety in a behavioral sensitization to nicotine regimen in the LRHR rats.

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1. Introduction

We have previously shown that the novelty-seeking phenotype in the outbred rat has a predictive value for individual differences in the expression of locomotor sensitization to nicotine [2,5,6]. The phenotype is determined in an experimentally-naïve outbred population of rats by the level of locomotor reactivity to a novel environment. Some rats exhibit high locomotor response to novelty (i.e., top 1/3rd of the population) and are identified as high responders, HRs; whereas some display low locomotor response to novelty (i.e., bottom 1/3rd of the population) and are identified as low responders, LRs. Chronic intermittent nicotine training followed by 1 wk of abstinence and a low dose nicotine challenge results in robust expression of locomotor sensitization to nicotine in HRs but not LRs, and this behavior is associated with distinct, phenotype-specific morphological and neurochemical alterations [2,5,6], validating this model as a tool for studying individual differences in behavioral and neural mechanisms that mark vulnerability to nicotine.

Present study aims to further elaborate on behavioral and neurobiological underpinnings of such vulnerability to nicotine in the HR rats with an emphasis on identifying therapeutic molecular targets. Withdrawal from drugs of abuse has been associated with a negative affective state such as anxiety, which may significantly contribute to maintenance of drug addiction [23]. In the laboratory rats, psychostimulant withdrawal-induced anxiety-like behavior is shown to involve augmentation of the brain stress system mediated by CRF in the central nucleus of the amygdala [CeA; 9,27,37]. Furthermore dysregulations in NPY signaling have been reported during development of dependence in the amygdala [27], where NPY and CRF produce opposing actions to conserve a balanced emotional state [15]. Although alterations in central NPY signaling in anxiety-like behavior following alcohol withdrawal has been extensively studied [12,34,35,47,49,54], the role of NPY signaling in

Abbreviations: BLA, basolateral nucleus of the amygdala; CeA, central nucleus of the amygdala; CRF, corticotropin releasing factor; CORT, corticosterone; EPM, elevated plus maze; HR, high responder; LR, low responder; LDB, light dark box; MeA, medial nucleus of the amygdala; NPY, neuropeptide Y; SI, social interaction; Y1R, Y1 receptor; Y2R, Y2 receptor.

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psychostimulant, specifically nicotine, abstinence-related negative affect is largely unexplored. We have recently shown that intermittent nicotine training followed by 1 wk of abstinence induces social anxiety-like behavior in HRs and a deficit in NPY signaling in the hippocampus and the amygdala along with increased CRF mRNA levels in the CeA [2]. Our findings are in confirmation of another report showing that nicotine abstinence leads to a long-lasting increase in anxiety-like behavior and a decrease in ratio of NPY to CRF in the amygdala [46]. All together, these findings suggest that neurobehavioral changes associated with nicotine exposure may be related to allostatic changes in stress (e.g., CRF) and anti-stress (e.g., NPY) neuropeptide systems [46]. In addition to amygdala, hippocampal NPY is also implicated in anxiolytic effects in traditional anxiety paradigms such as the elevated plus maze (EPM) [48]. In our hands, the hippocampus is implicated in the expression of behavioral sensitization to nicotine in the HR rats [5,6], and a deficit in the hippocampal NPY is shown following intermittent nicotine training and 1 wk of abstinence in the HR phenotype [2]. However, a causal relationship between the nicotine-associated neuropeptidergic (i.e., NPY and CRF) changes and abstinence-related anxiety-like behavior has not been established.

The biological effects of NPY are mediated by five classes of receptors with Y1 and Y2 being the major subtypes expressed in the rat brain [20]. The postsynaptic Y1 receptor is found in brain regions such as the hippocampus, the hypothalamus, and the amygdala where its activation is associated with neurobiological responses to ethanol [33,42] and psychostimulants [19,39]. Unlike Y1 receptors, Y2 receptors are located presynaptically and support a virtual antagonism to Y1 receptors [36]. Activation of Y2 receptors on NPY-ergic terminals inhibits release of endogenous NPY [22], hence decrease availability of NPY in the synaptic cleft. Originally, Nakajima et al. demonstrated anxiogenic-like effects of Y2 receptors in the EPM [32]. Antagonizing central Y2 receptors results in decreased operant responding for ethanol in animals with a history of alcohol dependence, and reverses the anxiogenic effects of withdrawal from a single dose of alcohol on the EPM [10,38,49]. Unlike Y1 receptors, Y2 receptors have not been studied within the context of behavioral effects of psychostimulants.

Present study investigates the effects of daily systemic administration of a novel, brain-penetrant Y2 receptor antagonist, JNJ-31020028, during 1 wk of abstinence in an intermittent behavioral sensitization to nicotine regimen on anxiety-like profile and locomotor sensitization to the low dose nicotine challenge in the LRHR rats, and subsequent neuropeptidergic effects on the CRF and the NPY (Y1R, Y2R) levels in the amygdala and the hippocampus. We hypothesize that the expression of locomotor sensitization to nicotine develops in the HR phenotype in conjunction with emergence of social anxiety-like behavior, a dysregulation in the amygdalar stress (e.g., CRF) and anti-stress (e.g., NPY) systems, along with a deficit in the Y2R in the hippocampus. Furthermore, Y2R antagonist is hypothesized to reverse these behavioral and neuropeptidergic adaptations in the nicotine vulnerable HR phenotype. We will use the novel Y2R antagonist agent, JNJ-31020028, which is recently described as selective against Y1, Y4 and Y5 receptors with the ability to penetrate into the brain and dose dependently occupy Y2R binding sites upon systemic delivery [44].

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. The Y2R antagonist, JNJ-31020028, was generously donated by Johnson & Johnson Pharmaceutical Research and Development, L.L.C., and dissolved in 20% 2-hydroxypropyl-beta-cyclodextrin.

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. A grand total of 234 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 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; $n = 78$) or LRs (i.e., rats that exhibited locomotor scores in the lowest third of the sample $n = 78$).

2.3. Anxiety paradigms

Animals were individually tested on all three anxiety tests (5 min each), and the order of testing was counterbalanced.

2.3.1. Elevated plus maze (EPM)

The apparatus was constructed of black-painted Plexiglas, with four elevated arms (70 cm from the floor, 45-cm long and 12-cm wide). The arms were arranged in a cross, with two opposite arms being enclosed by 45-cm high walls. The two other arms were open, having at their intersection a central 12×12 cm square platform giving access to all arms. The illumination above the central platform was 85 lux. Each rat was placed in the central square facing an open arm, and the time spent (with the four paws) in every arm, number of open arm entries and total number of arm entries were recorded for 5 min.

2.3.2. Light dark box (LDB)

The test was conducted in a $30 \times 60 \times 30$ cm Plexiglas shuttle box that was divided into two equal size compartments by a wall with an open door. One compartment was brightly illuminated while the other compartment was painted black with very dim light. The rats' locomotor activity and time spent in each compartment were monitored for 5 min.

2.3.3. Social interaction test (SI)

Testing took place in an open topped, rectangular, transparent social interaction box. The resident rat was placed in the box 8 min prior to placement of the experimental rat. The resident rat was of similar weight that was housed under identical conditions as the experimental rat, which had no previous contact with the experimental rat. The amount of time the experimental rat spent initiating social interaction (i.e., grooming, sniffing, following, crawling over or under) with the resident was determined for 5 min.

2.4. Experiment 1: Baseline LRHR differences in anxiety-like behavior, mRNA expressions of CRF, NPY, Y1R and Y2R in the amygdala and the hippocampus

Thirty-six male Sprague-Dawley rats (PN 22), following phenotype screening into LRs and HRs ($n = 12$/group) as described above, were allowed to rest for 22 days to age match the animals used in Experiment 2, handled twice a wk and on PN 44 were tested on the LDB and the SI tests as described above; and upon completion of testing, were rapidly decapitated. Brain tissue was harvested, sectioned and processed for in *situ* hybridization histochemistry as described below.

2.5. Experiment 2: Effects of systemic Y2R antagonism on nicotine-related behavioral and neuropeptidergic adaptations in the LRHR rats

One hundred and forty four male Sprague-Dawley rats (PN 22) were allowed to rest until PN 28 after phenotype screening, and were assigned to saline (1 ml/kg; s.c.) or nicotine (0.35 mg/kg; s.c.) training 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-day interval. Following the fourth training injection, rats were further assigned to vehicle (1 ml/kg, i.p.) or JNJ-31020028 (20 mg/kg, i.p.) therapeutic injection groups, and underwent 1 wk of nicotine-free period where they received daily vehicle or JNJ-31020028 injections. At the end of the abstinence period, all LRHR rats were challenged either with saline (1.0 ml/kg, s.c.) or with a low dose of nicotine (0.1 mg/kg, s.c.), and their locomotor response was monitored for 45 min Experimental groups and corresponding sample sizes are summarized in Table 1. Upon completion of the challenge session, all animals were