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The trace amine-associated receptor 1 agonists RO5263397 and RO5166017 attenuate quinpirole-induced yawning but not hypothermia in rats

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Abstract

Increasing evidence suggests that the trace amine-associated receptor 1 (TAAR1) is an important modulator of the dopaminergic system. Existing molecular evidence indicates that TAAR1 regulates dopamine levels via interactions with dopamine transporters (DAT) and D2 receptors. However, investigations to date have not been exhaustive and other pathways may be involved. In this study, we used a well-described set of behaviors, quinpirole-induced yawning and hypothermia, to explore the potential interaction of TAAR1 and D3 receptors, which are a member of the “D2-like” dopamine receptor subfamily. Previous studies have shown that for D2/D3 receptor agonists, the induction of yawning is a D3 receptor-mediated effect, whereas the inhibition of yawning and induction of hypothermia are D2 receptor-mediated effects. Quinpirole produced an inverted U-shaped dose-effect curve for yawning, which was shifted downward dose-dependently by each of the TAAR1 agonists RO5263397 and RO5166017. Quinpirole also produced dose-dependent hypothermia, which was not affected by either TAAR1 agonist. These results suggest that TAAR1 agonists may interact with D3 receptors and/or its downstream pathways, as opposed to D2 receptors. These findings may shed light on a previously unexplored possibility for the mechanism of TAAR1-mediated effects.

Keywords

TAAR1; yawning; hypothermia; quinpirole; rat

Introduction

The trace amine-associated receptor 1 (TAAR1) is a G protein-coupled receptor that has been identified as an important modulator of the dopaminergic system (Borowsky et al., 2001; Bunzow et al., 2001). As such, TAAR1 has relevance to a range of dopamine-related neuropsychiatric diseases including drug addiction, Parkinson’s disease, and schizophrenia (Berry, 2007; Wolinsky et al., 2007; Thorn et al., 2014). Existing molecular evidence

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Conflicts of interest

The authors declare that they have no competing interests.

suggests that TAAR1 regulates dopamine levels via interactions with dopamine transporters (DAT), D2 receptors, and related downstream signaling pathways (Xie and Miller, 2007; Xie et al., 2007; Harmeier et al., 2015). Despite these findings from *in vitro* and *ex vivo* studies, no investigations have implicated the involvement of D3 receptors in TAAR1-mediated effects. D3 receptors are part of the “D2-like” subfamily of dopamine receptor and signal through similar primary pathways as D2 receptors albeit with a more limited distribution in the brain restricted mainly to the limbic system (Levesque et al., 1992; Landwehrmeyer et al., 1993). Given the close relationship between D2 and D3 receptors and the important role that D3 receptors play in several neuropsychiatric disorders like drug addiction, Parkinson’s disease, and schizophrenia (Pich and Collo, 2015; Maramai et al., 2016; Sokoloff and Le Foll, 2017), it is possible that interactions between TAAR1 and D3 receptors have been overlooked.

This study employed a set of behaviors that have been well-characterized to distinguish D2 from D3 receptor activation *in vivo*. Administered systemically in rats, low doses of a D2/D3 receptor agonist such as quinpirole induce yawning behavior, whereas higher doses suppress yawning behavior and induce hypothermia (Collins et al., 2007). D2-preferring antagonists shift the descending limb of the yawning dose-effect curve rightward and inhibit hypothermia, whereas D3-preferring antagonists shift the ascending limb of the yawning dose-effect curve downward and do not inhibit hypothermia (Chaperon et al., 2003; Collins et al., 2005). Thus, the ascending limb of the yawning dose-effect curve is D3 receptor-mediated whereas the descending limb and hypothermia are D2 receptor-mediated. In the present study, a TAAR1 agonist (RO5263397 or RO5166017) was administered prior to quinpirole to determine the differential effects of TAAR1 activation on D2 and D3 receptor-mediated behaviors.

Methods

Subjects

Forty-eight adult male Sprague-Dawley rats (Harlan Sprague-Dawley Inc., Indianapolis, IN), at least 10 weeks old and weighing at least 300 g were housed under conditions described previously (Siemian et al., 2016). Water and standard rodent chow were always available except during testing. Animals were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use Committee, University at Buffalo, the State University of New York (Buffalo, NY), and with the 2011 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences, Washington, DC). Rats were handled and habituated to the rectal temperature measurements for two days before testing.

Procedure

Yawning behavior was defined as a prolonged (~1 s) opening of the mouth followed by rapid closure (Collins et al., 2005). On the test day, rats (n = 8 per treatment group) were placed into an empty rodent housing cage and allowed to habituate for 30 min. TAAR1 agonist or vehicle was administered 30 min prior to quinpirole (0.0032–0.32 mg/kg), which was administered using a cumulative dosing procedure. Observations of yawning began 20 min

after each injection, and the total number of yawns was recorded for a 10 min period thereafter. At the end of the 10 min observation, body temperature was measured by inserting a lubricated thermal probe (Physitemp Instruments Inc., Clifton, NJ) approximately 5 cm into the rectum prior to giving the next injection.

Drugs

Quinpirole dihydrochloride (Sigma-Aldrich, St. Louis, MO) was dissolved in normal saline. RO5263397 and RO5166017 (Research Triangle Institute) were administered in a vehicle of 1 part 190 proof ethanol, 1 part Alkamuls EL-620 (Rhodia, Cranbury, NJ) and 18 parts normal saline. All drugs were administered intraperitoneally in a volume of 1–2 ml/kg.

Data Analyses

GraphPad Prism 5.0 software was used for all statistical comparisons (GraphPad Software Inc., San Diego, CA). Results are expressed as the mean ($\pm$ SEM) number of yawns during the 10-min observation periods and as the mean ($\pm$ SEM) change in body temperature from baseline, both plotted as a function of quinpirole dose. Effects of quinpirole and each TAAR1 agonist were determined by two-way repeated measures ANOVA (treatment $\times$ quinpirole dose), with quinpirole dose entered as a within-subject factor, followed by Bonferroni's *post hoc* test. For all tests, $p < 0.05$ was considered significant.

Results

Rats rarely yawned after vehicle administration. Two-way repeated measures ANOVA revealed a significant RO5263397 $\times$ quinpirole interaction ($F(15, 140) = 2.68, p < 0.01$) with significant main effects of RO5263397 dose ($F(3, 140) = 9.10, p < 0.001$) and quinpirole dose ($F(5, 140) = 23.06, p < 0.001$). Bonferroni's *post hoc* tests revealed that 0.032 and 0.1 mg/kg quinpirole significantly increased yawning in the vehicle pretreatment group. Bonferroni's *post hoc* tests also revealed significant effects from 3.2 mg/kg RO5263397 at 0.1 mg/kg quinpirole and from 5.6 and 10 mg/kg RO5263397 at 0.032 and 0.1 mg/kg quinpirole as compared to vehicle pretreatment (Fig. 1, upper left). Two-way repeated measures ANOVA also revealed a significant RO5166017 $\times$ quinpirole interaction ($F(10, 105) = 2.14, p < 0.05$) with significant main effects of RO5166017 dose ($F(2, 105) = 5.52, p < 0.05$) and quinpirole dose ($F(5, 105) = 20.43, p < 0.0001$). Bonferroni's *post hoc* tests revealed that 0.032 and 0.1 mg/kg quinpirole significantly increased yawning in the vehicle pretreatment group. Bonferroni's *post hoc* tests also revealed significant effects from 3.2 mg/kg RO5166017 at 0.1 mg/kg quinpirole and from 10 mg/kg RO5166017 at 0.032 and 0.1 mg/kg quinpirole as compared to vehicle pretreatment (Fig. 1, upper right).

After vehicle administration, rats had a body temperature of 37.8 ± 0.1 °C. Two-way repeated measures ANOVA revealed a significant main effect of quinpirole dose ($F(5, 140) = 336.70, p < 0.0001$) but not of RO5263397 dose ($F(3, 140) = 1.96, NS$), with no significant RO5263397 $\times$ quinpirole interaction ($F(15, 140) = 0.77, NS$). Bonferroni's *post hoc* tests revealed that 0.032, 0.1, and 0.32 mg/kg quinpirole significantly decreased body temperature in the vehicle pretreatment group (Fig. 1, lower left). Two-way repeated measures ANOVA also revealed a significant main effect of quinpirole dose ($F(5, 105) = 313.24, p < 0.001$) but

not of RO5166017 dose ($F(2, 105) = 0.14$, *NS*), with no significant RO5166017 $\times$ quinpirole interaction ($F(15, 105) = 1.19$, *NS*). Bonferroni's *post hoc* tests revealed that 0.032, 0.1, and 0.32 mg/kg quinpirole significantly decreased body temperature in the vehicle pretreatment group (Fig. 1, lower right).

Discussion

Emerging evidence suggests that TAAR1 agonists may represent promising candidate treatments for neuropsychiatric disorders that involve aberrant dopamine functions such as drug addiction, Parkinson's disease, and schizophrenia. However, the mechanisms underlying TAAR1 modulation of the dopamine system are not yet fully understood. Existing *in vitro* and *ex vivo* data suggest that TAAR1 interacts with DAT and D2 receptors to affect the dopamine system (Xie and Miller, 2007; Xie et al., 2007; Grandy et al., 2016). However, in several investigations that reported changes in D2 receptor levels in TAAR1-knockout or -overexpressing animals, the "D2 receptor-selective" ligands used (e.g., raclopride, spiperone, domperidone) in fact label both D2 and D3 receptors with high affinity (Malmberg et al., 1993; Barone, 1999; Wolinsky et al., 2007; Espinoza et al., 2011, 2015; Newton et al., 2016). D2 receptor mRNA levels were also altered, but D3 receptor mRNA was not analyzed. Being "D2-like" receptors, D2 and D3 receptors share high sequence homology and function through similar primary pathways (Sibley and Monsma, 1992), thus it is possible that previous investigations have overlooked potential interactions between D3 receptors and TAAR1.

Consistent with previous findings (Collins et al., 2007; Baladi et al., 2011), in the present study quinpirole induced an inverted U-shaped dose-effect curve for yawning behavior and dose-dependent hypothermia. Both TAAR1 agonists dose-dependently shifted the yawning dose-effect curve downward but did not affect the hypothermic dose-effect curve. This pattern of results indicates that TAAR1 agonists influence D3 receptor- but not D2 receptor-mediated behaviors (Chaperon et al., 2003; Collins et al., 2005). Since synthetic TAAR1 ligands do not directly bind to any of the dopamine receptors (Revel et al., 2013), it is more likely that the downstream signaling pathways of D2 and D3 receptors are differentially modulated by TAAR1 activation.

Unlike D2 receptors which are expressed at high levels in many brain areas, D3 receptors have a more limited distribution with high enrichment in the limbic system (Levesque et al., 1992; Landwehrmeyer et al., 1993), and appear to have relevance to several diseases for which TAAR1 is also implicated. For example, preclinical studies have demonstrated that similar to TAAR1, D3 receptors are involved in drug intake and reward, and reinstatement of drug-seeking behavior (Heidbreder et al., 2005; Joyce and Millan, 2005). Of particular note, both TAAR1 agonists and D3 receptor antagonists reduce drug self-administration under schedules of high response requirements (Le Foll et al., 2005; Heidbreder and Newman, 2010; Thorn et al., 2014). Further, TAAR1 agonists and D3 receptor antagonists have been reported to have therapeutic potential for schizophrenia, whereas TAAR1 antagonists and D3 receptor agonists have shown therapeutic potential for Parkinson's disease (Revel et al., 2013; Pich and Collo, 2015; Pei et al., 2016). Coupled with data from the current study, it

appears that TAAR1 activation may produce behavioral effects, at least in part, by indirectly modulating D3 receptor function.

In conclusion, these results indicate that the TAAR1 agonists RO5263397 and RO5166017 reduce a D3 receptor-mediated behavior but do not affect D2 receptor-mediated behaviors induced by quinpirole in rats. Based on these *in vivo* data, future molecular investigations regarding the mechanism of TAAR1 should consider the potential involvement of D3 receptors.

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Figure 1.

Effects of the TAAR1 agonists RO5263397 (*left panels*) or RO5166017 (*right panels*) on quinpirole-induced yawning (*upper panels*) and hypothermia (*lower panels*) in rats. Vertical axes are yawns per 10 min (*upper panels*) and change in body temperature from baseline (*bottom panels*). Horizontal axes are the doses of quinpirole in mg/kg.