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REGULAR RESEARCH ARTICLE

Cariprazine Exhibits Anxiolytic and Dopamine D_3 Receptor-Dependent Antidepressant Effects in the Chronic Stress Model

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Abstract

Background: Cariprazine, a D_3-preferring dopamine D_2/D_3 receptor partial agonist, is a new antipsychotic drug recently approved in the United States for the treatment of schizophrenia and bipolar mania. We recently demonstrated that cariprazine also has significant antianhedonic-like effects in rats subjected to chronic stress; however, the exact mechanism of action for cariprazine's antidepressant-like properties is not known. Thus, in this study we examined whether the effects of cariprazine are mediated by dopamine D_3 receptors.

Methods: Wild-type and D_3-knockout mice were exposed to chronic unpredictable stress for up to 26 days, treated daily with vehicle, imipramine (20 mg/kg), aripiprazole (1 and 5 mg/kg), or cariprazine (0.03, 0.1, 0.2, and 0.4 mg/kg), and tested in behavioral assays measuring anhedonia and anxiety-like behaviors.

Results: Results showed that cariprazine significantly attenuated chronic unpredictable stress-induced anhedonic-like behavior in wild-type mice, demonstrating potent antidepressant-like effects comparable with aripiprazole and the tricyclic antidepressant imipramine. This antianhedonic-like effect of cariprazine was not observed in D_3-knockout mice, suggesting that the cariprazine antidepressant-like activity is mediated by dopamine D_3 receptors. Moreover, cariprazine significantly reduced drinking latency in the novelty-induced hypophagia test in wild-type mice, further confirming its antianhedonic-like effect and showing that it also has anxiolytic-like activity.

Conclusions: In combination with previous studies, these results suggest that cariprazine has a unique pharmacological profile and distinct dopamine D_3 receptor-dependent mechanism of action that may be beneficial in the treatment of schizophrenia, bipolar disorder, and major depressive disorder.

Keywords: cariprazine, antidepressant, anxiolytic, dopamine D_3 receptor, anhedonia

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Significance Statement

In a mouse model of depression, cariprazine produces antidepressant-like activity through action on dopamine D_3 receptors. Indeed, cariprazine reversed anhedonia-like and anxiety-like deficits in this chronic stress model. These results suggest that cariprazine has a unique and distinct dopamine D_3 receptor-dependent mechanism of action that may be beneficial in the treatment of schizophrenia, bipolar disorder, and major depressive disorder.

Introduction

Major depressive disorder is a common, chronic, recurring illness associated with significant functional and social impairment (Duric and Duman, 2013). Effective treatment of depression is challenging, with most patients failing to achieve remission after initial antidepressant treatment. As a result, antipsychotics are increasingly used as adjunct therapy to enhance antidepressant efficacy, especially since augmentation with atypical agents improves pharmacological and side effect profiles (Nelson and Papakostas, 2009). Although central nervous system mechanisms underlying antidepressant actions of atypical antipsychotics are still unclear, it has been proposed that a compound that exhibits high affinity and occupancy of both D_2 and D_3 dopamine receptors may be effective as a treatment for depressive disorders as well as the negative symptoms of schizophrenia. A dopamine D_3 receptor strategy for treatment of depression or schizophrenia is based on the brain distribution and the putative role of D_3 receptors (Gross and Drescher, 2012); these receptors have the highest density in rat and human ventral striatum (Sokoloff et al., 1990; Gurevich and Joyce, 1999), one of the core areas implicated in the pathology of schizophrenia as well as depression and anxiety. The potential involvement of dopamine D_3 receptors in the effects of antidepressants was suggested by several preclinical studies demonstrating that administration of antidepressant drugs enhances D_3 receptor gene expression and/or binding in distinct brain regions (Maj et al., 1998; Lammers et al., 2000).

Cariprazine, a novel, orally active antipsychotic agent that is a potent dopamine D_2/D_3 receptor partial agonist with preferential binding to D_3 receptors, was recently approved by the U.S. Food and Drug Administration for the treatment of both schizophrenia and acute manic or mixed episodes associated with bipolar I disorder (Ágai-Csongor et al., 2012; Veselinovic et al., 2013; Findlay et al., 2016). Among atypical antipsychotics, cariprazine displays the highest D_3 receptor binding affinity and D_3 vs. D_2 receptor selectivity (by approximately 6- to 8-fold) (Kiss et al., 2010; McCormick et al., 2010; Ellenbroek and Cesura, 2015). These features could be responsible for cariprazine showing a more balanced dopamine D_2 and D_3 receptor brain occupancy in vivo in both rodents (Gyertyán et al., 2011; Kiss et al., 2012) and patients (Girgis et al., 2016) compared with other antipsychotics, which displayed preferential occupancy for D_2 vs. D_3 receptors (Graff-Guerrero et al., 2009; McCormick et al., 2010; Mizrahi et al., 2011). Furthermore, cariprazine was also found to be unique among antipsychotics in its ability to increase dopamine D_3 receptor levels in D_3 receptor-rich brain regions following chronic treatment (Choi et al., 2014). The nucleus accumbens shell is one of the brain regions of particular interest where cariprazine produced this effect, and an increase in D_3 receptor expression in this region has been proposed to be a common neurobiological mechanism of antidepressant treatments (Lammers et al., 2000).

Cariprazine has demonstrated antidepressant-like activity in the chronic mild stress model in rats and was shown to reduce anhedonia-like behavior, a hallmark symptom of depression and negative symptoms of schizophrenia (Papp et al., 2014); however, the mechanism of action underlying cariprazine's antidepressant-like effects has not been elucidated. A previous study of cariprazine in dopamine D_3 receptor-knockout (D_3-KO) mice showed that cariprazine has D_3 receptor-dependent positive/protective effects on cognitive function, suggesting that the efficacy of cariprazine in some symptom domains is at least partly mediated through its D_3-receptor activity (Zimnisky et al., 2013). Therefore, in the current study, we investigated the antidepressant- and anxiolytic-like effects of cariprazine in a mouse model of chronic unpredictable stress (CUS) and determined whether these effects are mediated by D_3 receptors using mutant D_3-KO mice. Moreover, we explored whether there were any differences in the antidepressant- and anxiolytic-like effects of cariprazine compared with aripiprazole, another atypical antipsychotic and dopamine receptor partial agonist, which, unlike cariprazine, has a higher affinity for dopamine D_2 vs. D_3 receptors.

Materials and Methods

Animals

Adult male C56BL/6 mice (Jackson Laboratory) were used for all studies, except for the experiment using mutant D_3-KO and littermate wild-type (WT) mice obtained from Dr. Marc Caron (Duke University, Durham, NC) (Beaulieu et al., 2007). Mice were housed in groups of 1 to 5 per cage under a 12-h-light/-dark cycle at constant temperature (25°C) and humidity with ad libitum access to food and water (except when indicated). Prior to any treatments or experiments, animals were allowed at least 1 week of habituation to the housing conditions. All mice were age and weight matched (~25–30 g) at the time of the first stressor. The maintenance of mouse colonies and all animal treatments and procedures were in strict accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the Yale Animal Care and Use Committee.

Chronic Unpredictable Stress

Mice were exposed to a variable sequence of unpredictable stressors as described in our recent studies (Koo and Duman, 2008; Duric et al., 2010; Koo et al., 2010). Overall, mice were subjected to 10 different stressors, 2/d for up to 26 consecutive days. Stressors included: restraint stress (1 hour), cold (1 hour), forced swim (10 minutes), light/dark cycle disturbance, strobe light, odor, no/wet bedding, and cage tilt. Control mice were handled daily during vehicle (e.g., Tween 80 [1%]) administration and weighing. In conjunction with CUS exposure, imipramine (20 mg/kg; q.d.), aripiprazole (1 and 5 mg/kg; b.i.d.), or cariprazine (0.03, 0.1, 0.2, and 0.4 mg/kg; b.i.d.) were administered i.p. for 26 days (n = 6–8 mice/group). Twice-daily (i.e., b.i.d.) dosing regimen was used for cariprazine due to its relatively short plasma $t_{1/2}$ of approximately 3.2 hours. This was based on our previous pharmacokinetic studies in male mice (26–32 g) administered (i.p.) 1 mg/kg

of cariprazine (data on file, G. Richter Plc). Moreover, vehicle was administrated i.p. to the control vehicle group (b.i.d.) and the imipramine group once per day to standardize the total number of injections per animal. Behavioral testing started after 18 days of CUS with continued exposure to stress and drug treatments. Each test was performed a minimum of 12 hours after the last stressor and last injection.

Behavioral Testing

Sucrose and Water Consumption Tests

The effects of CUS and drug administration on anhedonic-like behavior were assessed by measuring sucrose consumption (Pothion et al., 2004) as previously described in our laboratory (Duric et al., 2010). Mice were single-housed and habituated to palatable 1% sucrose solution (Sigma) for 48 hours (free access) prior to testing. Following overnight fluid deprivation, animals were exposed to sucrose solution for 1 hour, and the total volume consumed was measured by weight and recorded. Results are expressed in grams of sucrose consumed during a 1-hour test period. For the water consumption test, the same procedure was repeated with tap water. Both tests were conducted after at least 18 days of consecutive CUS exposure.

Novelty-Induced Hypophagia Test

Mice were habituated with diluted (1:3 milk/water) sweetened condensed milk (Carnation, Nestle USA) for 1 hour on 3 consecutive days. At first, mice were tested in the home cage under normal lighting by replacing the water bottle with the milk (1 mL) presented in a small petri dish placed in the middle of the cage, and the latency to drink was recorded with a maximum cutoff set at 5 minutes. For novel cage testing, latency to drink was recorded after mice were placed in different clean cages of the same dimensions (no bedding) under dim lighting (~50 lux) with white paper under cages to enhance aversion. Animals with latency to drink exceeding 10 minutes in the home cage were excluded from the novelty-induced hypophagia analysis.

Locomotor Activity Test

Locomotor activity was measured during the CUS treatment paradigm to control for changes in ambulatory activity. Mice were placed in novel home cage-like arenas, and their behavior was video-recorded over the next 30 minutes. Total locomotor activity was determined as distance traveled (in meters) using the video-tracking software ANY-maze (Stoelting Co).

Statistical Analysis

One-way ANOVA analysis (StatView 5.0; SAS Institute) was used to determine whether CUS produced significant effects on sucrose consumption or drinking latency in the novelty-induced hypophagia test. Since imipramine was used as a positive control and the low dose of cariprazine was not tested in D_3-KO mice, the experimental design was asymmetric. We excluded animal groups not represented in both genotypes for the 2-way ANOVA analysis testing for significant interactions between genotype and treatment for sucrose consumption, water consumption, locomotor activity, and drinking latency. All mouse groups were included in the posthoc 1-way ANOVA and pairwise comparison using Fisher's probable least-squares difference (PLSD).

Results

Cariprazine Produces Antidepressant- and Anxiolytic-Like Effects in CUS Mice

In the first phase of the study, we investigated the dose range of cariprazine required to reverse CUS-induced anhedonia-like and anxiety-like deficits in mice. Mice were exposed to a variable sequence of unpredictable stressors (2/d) for a total of 26 days (Figure 1a). Throughout the stress paradigm, mice were administered vehicle or cariprazine, or imipramine that served as positive control; there was also a separate group not exposed to the CUS procedure that received vehicle.

One-way ANOVA analysis revealed that 21 days of CUS exposure resulted in significant changes in sucrose consumption (day 21; $F_{5,35}$=30.28, P<.0001) (Figure 1b). Vehicle-treated mice subjected to CUS exhibited a significant decrease in sucrose consumption, indicating CUS-induced anhedonia-like deficits (P<.0001) (Figure 1b). In addition, treatments with cariprazine 0.2 mg/kg (but not 0.03 or 0.1 mg/kg) significantly attenuated the CUS-induced decrease of sucrose consumption (P<.0001 vs. CUS + vehicle; Figure 1b), demonstrating cariprazine's antidepressant-like activity. Likewise, chronic administration of imipramine (20 mg/kg) also robustly increased sucrose consumption in CUS animals (P<.0001 vs. CUS + vehicle; Figure 1b), which further validated the assay sensitivity to classical antidepressant treatment (Monleon et al., 1995; Elsayed et al., 2012; Papp et al., 2014). Analysis of water consumption (day 18; $F_{5,35}$=0.39, P=.85; Table 1) and locomotor activity (day 22; $F_{5,35}$=1.15, P=.35; Table 1) showed no significant difference between groups, indicating that effects of CUS on sucrose consumption were not due to experimental bias related to changes in drinking behavior or overall ambulatory activity, respectively.

The potential effects of cariprazine on CUS-induced anxiety-like behaviors were also addressed in the same cohort of mice using the novelty-induced hypophagia test. ANOVA analysis indicated no significant effect of CUS or drug treatment on latency to drink sweetened milk in home cage conditions (day 24; $F_{5,33}$=0.72, P=.61; data not shown). Conversely, ANOVA analysis showed significant effects of CUS or drug treatment when the same test was conducted in the novel arena (i.e., novelty-induced hypophagia test; day 25; $F_{5,35}$=6.41, P=.0003). Specifically, the posthoc analysis revealed that latency to drink was significantly increased in mice exposed to CUS, demonstrating an increase in anxiety-like behavior (P<.0001 vs. control; Fig 1c). Further posthoc analysis showed that cariprazine treatment robustly attenuated novelty-induced hypophagia, suggesting anxiolytic-like effects, as significant reductions in drinking latency were observed in CUS mice treated with both cariprazine 0.1 mg/kg (P=.003 vs. CUS + vehicle; Figure 1c) and 0.2 mg/kg doses (P=.0007 vs. CUS + vehicle; Figure 1c). Furthermore, the anxiolytic action of cariprazine was comparable with the anxiolytic response to imipramine (20 mg/kg), since we found significantly reduced drinking latency in CUS mice treated with imipramine (P=.0006 vs. CUS + vehicle) (Figure 1c).

Antidepressant Actions of Cariprazine Require Dopamine D_3 Receptors

To determine whether antidepressant-like properties of cariprazine are mediated through dopamine D_3 receptors, in the second phase of the study we examined if deletion of D_3 receptors influences the behavioral response to chronic stress (Figure 2a). Notably, previous reports have shown that D_3-KO mice do not exhibit baseline anxiety- or depressive-like behaviors (Chourbaji et al., 2008;

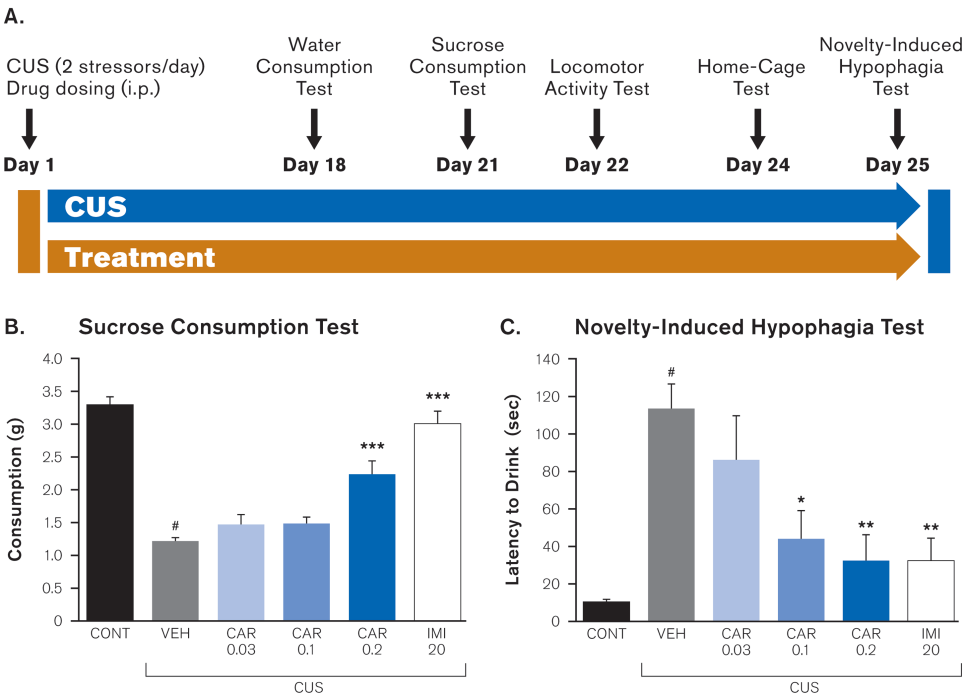

Figure 1. Influence of chronic unpredictable stress (CUS) and drug treatment on anhedonia- and anxiety-like behavioral responses. (A) Mice were exposed to the CUS paradigm or control conditions for 26 days and were administered vehicle, cariprazine, or imipramine. Sucrose consumption, water consumption, novelty-induced hypophagia, and locomotor activity were determined. Behavioral results for (B) sucrose consumption test (day 21 of CUS) and (C) novelty-induced hypophagia test (day 25 of CUS) are shown, expressed as mean ± SEM (n = 6–7). All doses are in mg/kg. #P < .0001 compared to nonstressed control group; *P < .05 compared with CUS + vehicle group; **P < .001 compared to CUS + vehicle group; ***P < .0001 compared with CUS + vehicle group (1-way ANOVA and Fisher's PLSD posthoc analysis). CAR, cariprazine; CONT, control; IMI, imipramine; VEH, vehicle.

Table 1. Influence of CUS and Drug Treatment on Water Consumption and Locomotor Activity in Wild-Type Mice

Treatment Group	Water Consumption Mean (g) ± SEM	Locomotor Activity Mean (m) ± SEM
Control (vehicle)	1.87 ± 0.11	35.1 ± 1.6
CUS + vehicle	1.95 ± 0.09	35.8 ± 4.7
CUS + CAR (0.03 mg/kg; b.i.d.)	2.01 ± 0.09	30.1 ± 1.6
CUS + CAR (0.1 mg/kg; b.i.d.)	1.96 ± 0.10	30.4 ± 2.0
CUS + CAR (0.2 mg/kg; b.i.d.)	2.03 ± 0.10	29.1 ± 1.4
CUS + IMI (20 mg/kg)	2.03 ± 0.10	30.6 ± 3.4

Abbreviations: b.i.d., twice a day; CAR, cariprazine; CUS, chronic unpredictable stress; IMI, imipramine.
There were no overall significant effects of CUS or drug treatment using ANOVA.

Leggio et al., 2008). CUS-induced decreases in sucrose consumption were observed in both D_3-KO and wild-type littermate mice, establishing the validity of the CUS model in D_3-KO mice and the lack of exacerbation of the anhedonia-like effects of CUS in D_3-KO mice (day 21; $F_{7,52}$=20.50, P<.0001; Figure 2b). Furthermore, 2-way ANOVA analysis showed a significant interaction between genotype and cariprazine treatment ($F_{2,42}$=18.74, P<.0001). Consistent with the initial experiment, cariprazine 0.2 mg/kg attenuated CUS-induced decreases in sucrose consumption (P<.0001 vs. CUS + vehicle; Figure 2b) in wild-type mice; the magnitude of this effect was similar to that observed with imipramine (P<.0001 vs. CUS + vehicle; Figure 2b). However, in D_3-KO mice, cariprazine did not reverse CUS-induced decreases in sucrose consumption at any dose (P=.56 vs. CUS + vehicle; Figure 2b), suggesting that the antidepressant-like activity of cariprazine required D_3 receptor activation. Finally, there were no significant effects of CUS, cariprazine treatment, or genotype on water consumption (day 22; $F_{7,52}$=1.90, P=.09; Table 2) or locomotor activity (day 22; $F_{7,52}$=1.19, P=.32; Table 2), indicating no difference in overall liquid consumption and ambulatory behavior between D_3-KO and wild-type mice.

As in the previous experiment, the effect of D_3 receptor deletion on anxiety-like behaviors was evaluated in the novelty-induced hypophagia test. In home cage conditions, 1-way ANOVA showed no significant effect of CUS on drinking latency (day 23; $F_{7,52}$=2.10, P=.06; data not shown), while 2-way ANOVA further revealed no main effect of cariprazine 0.2 mg/kg treatment ($F_{2,42}$=1.270, P=.29) or genotype ($F_{1,42}$=1.058, P=.31) but a significant genotype x treatment interaction ($F_{2,42}$=5.015, P<.01). In the novelty-induced hypophagia test, posthoc analysis confirmed that CUS induced a significant increase in latency to drink (day 24; $F_{7,52}$=3.60, P=.003) (Figure 2c). In addition, 2-way ANOVA analysis revealed a significant effect of cariprazine treatment ($F_{2,42}$=4.48, P=.017) and a significant interaction between the drug treatment and genotype ($F_{2,42}$=3.27, P=.048). In wild-type animals, negative effects of CUS on drinking latency were significantly inhibited by both cariprazine 0.2 mg/kg (P=.005 vs. CUS + vehicle; Figure 2c) and imipramine (P=.0008 vs. CUS + vehicle; Figure 2c) treatments. However, there were no significant effects of CUS or drug treatment in the D_3-KO mice when tested in novel cage conditions. D_3-KO mice not subjected to CUS exhibited elevated rearing and exploratory behavior. This "distracted" behavior explains the significantly higher latency to drink in D_3-KO when compared to WT controls (P=.03). Lastly, CUS-exposed D_3-KO animals showed similar latency to drink as D_3-KO control mice (Figure 2c). As a result, we confirmed the anxiolytic

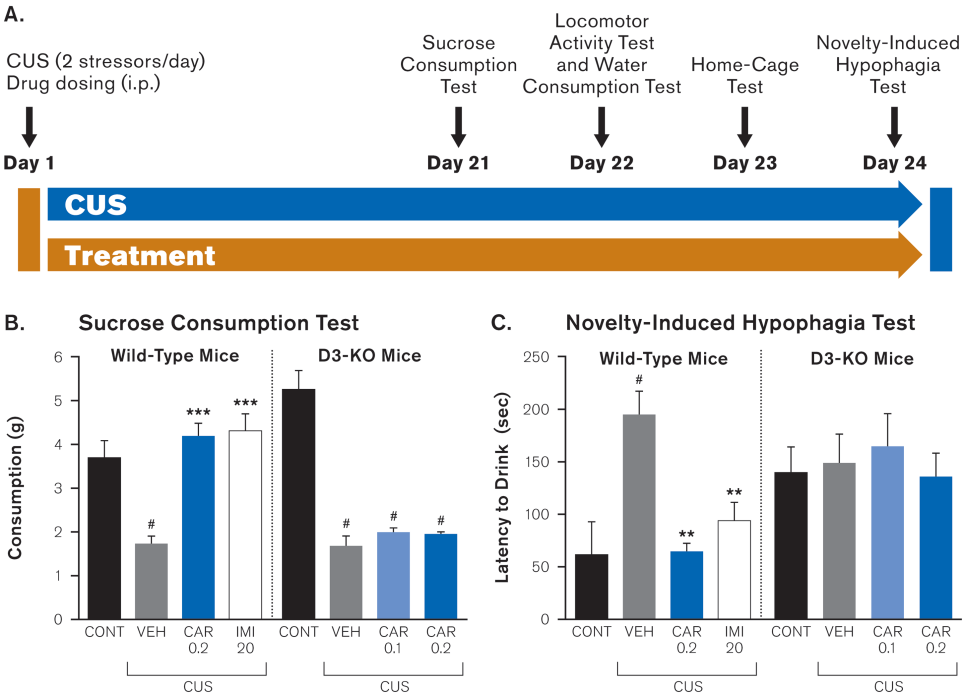

Figure 2. Influence of dopamine D_3 receptor deletion on cariprazine actions in behavioral models of depression. (A) Experimental paradigm for behavioral testing and CUS exposure of D_3 knockout mice (D_3-KO) and wild-type (WT) littermates (n = 6–8). Both genotypes were tested in the (B) sucrose consumption test (day 21 of chronic unpredictable stress [CUS]) and (C) novelty-induced hypophagia test (day 24 of CUS). All doses are in mg/kg. Results are expressed as mean ± SEM; #P < .0001 compared to non-stressed control group; **P < .01 compared to CUS + vehicle group; ***P < .0001 compared to CUS + vehicle group (1-way ANOVA and Fisher's PLSD posthoc analysis). CAR, cariprazine; CONT, control; IMI, imipramine; VEH, vehicle.

Table 2. Influence of CUS and Drug Treatment on Water Consumption and Locomotor Activity in the D_3 Knockout (D_3-KO) Mice and Wild-Type Littermates

Genotype	Treatment Group	Water Consumption Mean (g) ± SEM	Locomotor Activity Mean (m) ± SEM
Wild-type mice	Control (vehicle)	1.70 ± 0.07	34.3 ± 3.0
	CUS + vehicle	1.61 ± 0.05	39.7 ± 1.8
	CUS + CAR (0.2 mg/kg; b.i.d.)	1.64 ± 0.07	36.7 ± 2.7
	CUS + IMI (20 mg/kg)	1.45 ± 0.08	40.4 ± 2.2
D_3-KO mice	Control (vehicle)	3.15 ± 1.03	36.7 ± 2.8
	CUS + vehicle	1.76 ± 0.07	42.6 ± 2.4
	CUS + CAR (0.1 mg/kg; b.i.d.)	1.70 ± 0.14	41.1 ± 5.4
	CUS + CAR (0.2 mg/kg; b.i.d.)	1.73 ± 0.13	43.1 ± 3.0

Abbreviations: b.i.d., twice a day; CAR, cariprazine; CUS, chronic unpredictable stress; D_3-KO, dopamine D_3 receptor knockout mice; IMI, imipramine.
There were no overall significant effects of genotype, drug treatment, or CUS using ANOVA.

properties of cariprazine in the novelty suppressed feeding test but could not conclusively determine the involvement of dopamine D_3 receptors in this effect since the D_3-KO mice exhibited this particular behavior and experimental confound.

Comparison of Antidepressant and Anxiolytic Actions of Cariprazine and Aripiprazole

We compared the antidepressant and anxiolytic actions of cariprazine to aripiprazole in mice exposed to the CUS paradigm or control conditions (Figure 3a). In wild-type mice exposed to CUS, both cariprazine and aripiprazole demonstrated robust antidepressant-like (day 21; $F_{5,42}$=8.41, P<.0001; Figure 3b) and anxiolytic-like (day 21; $F_{5,41}$=9.68, P<.0001; Figure 3c) effects in sucrose consumption and novelty-induced hypophagia tests, respectively. Moreover, cariprazine significantly attenuated CUS-induced anhedonia-like behavior at both 0.2 mg/kg (P=.018 vs. CUS + vehicle) and 0.4 mg/kg (P=.0002 vs. CUS + vehicle), while aripiprazole was effective only at the higher 5 mg/kg dose (P<.0001 vs. CUS + vehicle) (Figure 3b). Mice treated with 5 mg/kg aripiprazole also exhibited a significant increase in water consumption (day 22; $F_{5,42}$=6.419; P<.001; posthoc Fisher's PLSD P=.0085 vs. control, P=.0009 vs. CUS + vehicle; Table 3), suggesting that changes in sucrose consumption observed following 5 mg/kg aripiprazole administration may, in part, be attributed to an overall increase in drinking behavior. Furthermore, no effects of cariprazine or aripiprazole were observed on the locomotor activity (Table 3).

Discussion

Cariprazine is a newly developed antipsychotic medication that was approved for the treatment of schizophrenia and manic

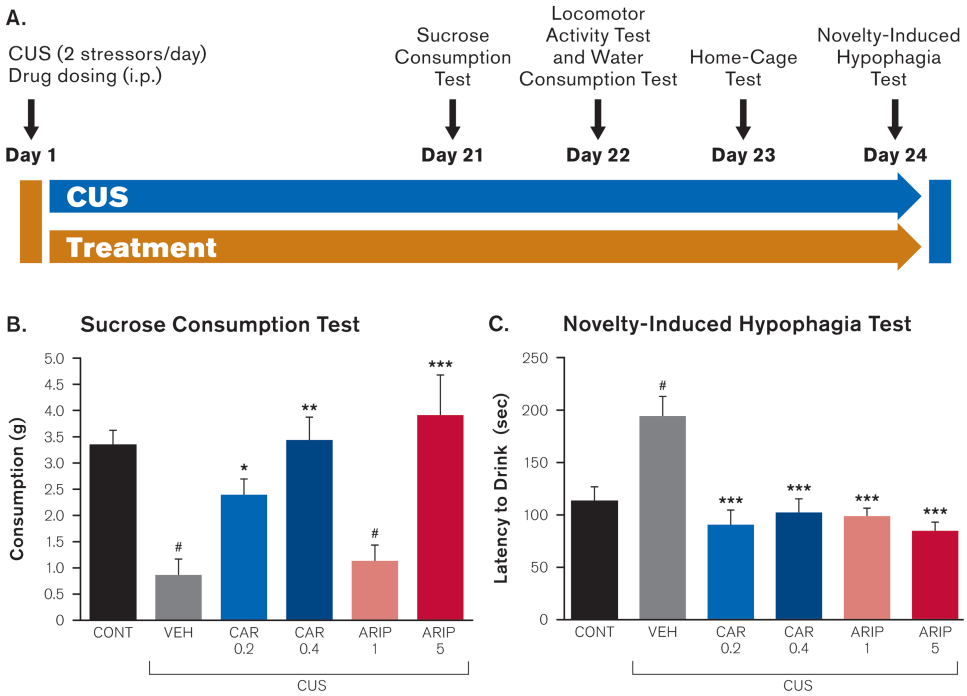

Figure 3. Antidepressant and anxiolytic pharmacological profile of cariprazine is compared with aripiprazole in the chronic unpredictable stress (CUS) model. (A) Mice were exposed to the CUS paradigm or control conditions for 26 days and were administered either vehicle, cariprazine or aripiprazole. The effects of drug treatment on (B) anhedonia- and (C) anxiety-like behavioral responses are shown. All doses are in mg/kg. Results are expressed as mean ± SEM; #P < .0001 compared to non-stressed control group; *P < .05 compared with CUS + vehicle group; **P < .001 compared with CUS + vehicle group; ***P < .0001 compared with CUS + vehicle group (1-way ANOVA and Fisher's PLSD posthoc analysis). ARIP, aripiprazole; CAR, cariprazine; CONT, control; VEH, vehicle.

Table 3. Effects of Cariprazine or Aripiprazole Treatments on Water Consumption and Locomotor Activity in Wild-Type CUS Mice

Treatment Group	Water Consumption Mean (g) ± SEM	Locomotor Activity Mean (m) ± SEM
Control (vehicle)	1.45 ± 0.23	17.7 ± 1.1
CUS + vehicle	1.23 ± 0.21	18.5 ± 0.8
CUS + CAR (0.2 mg/kg; b.i.d.)	0.84 ± 0.15	16.7 ± 1.9
CUS + CAR (0.4 mg/kg; b.i.d.)	0.94 ± 0.15	18.0 ± 1.1
CUS + ARIP (1 mg/kg; b.i.d.)	1.41 ± 0.26	18.0 ± 0.7
CUS + ARIP (5 mg/kg)	2.20 ± 0.12[a]	21.0 ± 1.1

Abbreviations: ARIP, aripiprazole; b.i.d., twice a day; CAR, cariprazine; CUS, chronic unpredictable stress.
ANOVA analysis showed statistically significant differences in the water consumption test ($F_{5,42} = 6.42$, $P < .001$).
[a]Posthoc analysis revealed significant difference when compared to control (vehicle) or CUS + vehicle groups.

or mixed episodes associated with bipolar I disorder based on results from a number of phase 2/3 clinical trials (Durgam et al., 2014, 2015a, 2015b; Calabrese et al., 2015; Kane et al., 2015; Sachs et al., 2015). Cariprazine is a partial agonist at both dopamine D_2 and D_3 receptors as well as serotonin 5-HT_{1A} receptors and in this regard is relatively similar to the currently available atypical antipsychotics, aripiprazole, and brexpiprazole. However, the multifunctional pharmacological properties of cariprazine at dopamine receptors, specifically its superior affinity and selectivity for D_3 receptors, differentiate it from most atypical antipsychotic agents (including compounds with partial agonist as well as those with full antagonist properties at D_2/D_3 receptors) (Ellenbroek and Cesura, 2015; Stahl, 2016).

Cariprazine is unique in its ability to bind D_3 receptors with higher affinity than even dopamine itself, which essentially results in blockade of D_3 receptors (Freedman et al., 1994; Sautel et al., 1995; Kiss et al., 2010). In contrast, most other atypical antipsychotics have relatively lower affinities for the D_3 receptor and in the presence of normal brain levels of dopamine are unable to block these receptors (Stahl, 2016). Furthermore, since D_3 receptors are thought to be involved in the regulation of mood, cognition, and motivation (Gross and Drescher, 2012), compounds like cariprazine that exhibit high affinity and occupancy of these receptors could also potentially be useful in the treatment of depression and the negative symptoms of schizophrenia. This has been further supported by our previous studies showing that cariprazine has potent antidepressant-like, antipsychotic-like, and procognitive effects in rodent models (Gyertyán et al., 2011; Zimnisky et al., 2013; Papp et al., 2014; Neill et al., 2016; Watson et al., 2016). Moreover, in recent clinical studies in patients suffering from schizophrenia with predominant negative symptoms, cariprazine provided significantly greater improvement than risperidone for both negative symptoms and functionality (Debelle et al., 2015). These results further support the differentiating features of cariprazine.

The antidepressant-like efficacy of cariprazine was demonstrated in a chronic mild stress model in rats by reducing anhedonic-like behavior (Papp et al., 2014); however neural mechanisms underlying these effects are still unclear. In the current study, cariprazine significantly attenuated CUS-induced sucrose consumption in a novel environment in wild-type mice, demonstrating antidepressant-like activity that was comparable with the tricyclic antidepressant imipramine. Interestingly, the same antianhedonic-like actions of cariprazine were not

observed in D_3-KO mice, indicating that dopamine D_3 receptors are required to mediate the antidepressant-like effects of cariprazine. Furthermore, previous studies in D_3-KO mice have suggested that blockade of D_3 receptors in the brain may produce anxiolytic effects (Steiner et al., 1997). In this study, we demonstrated that cariprazine is an effective anxiolytic in wild-type mice by significantly reducing latency to drink in the novelty-induced hypophagia test. However, D_3-KO mice not exposed to CUS showed elevated latency to drink in the novelty-induced hypophagia test, possibly due to distracted behavior displayed by D_3-KO control mice. Similar increases in rearing activity have been previously observed in D_3-KO mice (Yarkov et al., 2010). We found no effect of CUS exposure or drug treatment in D_3-KO mice, probably because of the elevated baseline exhibited in the D_3-KO animals. Although this experimental confound precluded the determination of clear involvement of D_3 receptors in the cariprazine effect in this test, we confirmed in 3 independent cohorts that cariprazine exerts anxiolytic properties and reverses the effect of chronic stress exposure.

The neuropharmacological profile of cariprazine is generally considered to be similar to aripiprazole, another atypical antipsychotic that is commonly used for the treatment of schizophrenia and bipolar disorder, except that cariprazine has a greater affinity and selectivity for D_3 vs. D_2 receptors (Tadori et al., 2011; Ellenbroek and Cesura, 2015; Findlay et al., 2016; Stahl, 2016). Aripiprazole also has been shown to be effective as an adjunct treatment for major depressive disorder (Bourin et al., 2009; Lenze et al., 2015; Zhou et al., 2015). Our results show that administration of a high dose of aripiprazole produced an antidepressant-like response, but also induced increases in total fluid consumption, which could be due to polydipsia and/or polyuria, potential side effects of some antipsychotic agents (Bersani et al., 2007; Meulendijks et al., 2010; Gandhi et al., 2016). In this regard, cariprazine has a more specific antidepressant-like effect that was independent of changes in overall liquid consumption. We found that both drugs have similar anxiolytic-like effects in the novelty-induced hypophagia test. The antidepressant- and anxiolytic-like effects of aripiprazole were not explored in D_3-KO mice because of the nonspecificity of its anhedonia-like actions and the difficulty in interpreting anxiolytic-like activity of drugs in D_3-KO animals.

Overall, in combination with previous studies, these data indicate that cariprazine has a unique pharmacological profile and, with its distinct dopamine D_3 receptor-preferring mechanism of action, may have potential efficacy in the treatment of depressive disorders and negative symptoms of schizophrenia.

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Statement of Interest

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