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The dopamine D_3-preferring D_2/D_3 dopamine receptor partial agonist, cariprazine, reverses behavioural changes in a rat neurodevelopmental model for schizophrenia

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Abstract
Current antipsychotic medication is largely ineffective against the negative and cognitive symptoms of schizophrenia. One promising therapeutic development is to design new molecules that balance actions on dopamine D_2 and D_3 receptors to maximise benefits and limit adverse effects. This study used two rodent paradigms to investigate the action of the dopamine D_3-preferring D_3/D_2 receptor partial agonist cariprazine. In adult male rats, cariprazine (0.03–0.3 mg/kg i.p.), and the atypical antipsychotic aripiprazole (1–3 mg/kg i.p.) caused dose-dependent reversal of a delay-induced impairment in novel object recognition (NOR). Treating neonatal rat pups with phencyclidine (PCP) and subsequent social isolation produced a syndrome of behavioural alterations in adulthood including hyperactivity in a novel arena, deficits in NOR and fear motivated learning and memory, and a reduction and change in pattern of social interaction accompanied by increased ultrasonic vocalisations (USVs). Acute administration of cariprazine (0.1 and 0.3 mg/kg) and aripiprazole (3 mg/kg) to resultant adult rats reduced neonatal PCP-social isolation induced locomotor hyperactivity and reversed NOR deficits. Cariprazine (0.3 mg/kg) caused a limited reversal of the social interaction deficit but neither drug affected the change in USVs or the deficit in fear motivated learning and memory. Results suggest that in the behavioural tests investigated cariprazine is at least as effective as aripiprazole and in some paradigms it showed additional beneficial features further supporting the advantage of combined dopamine D_3/D_2 receptor targeting. These findings support recent clinical studies demonstrating the efficacy of cariprazine in treatment of negative symptoms and functional impairment in schizophrenia patients.

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1. Introduction

Schizophrenia is a debilitating, lifelong psychiatric disorder affecting approximately 1% of the population. Current antipsychotics provide therapeutic benefit to the positive symptoms, but have relatively little impact on the negative (social withdrawal and anhedonia) and cognitive deficits (Keefe et al., 2007) which precede psychosis and show a stronger correlation to patient functional outcome (Green et al., 2004; Mintz and Kopelowicz, 2007). All current antipsychotics are dopamine D_2 receptor antagonists, a property thought to contribute to the reduction in positive symptoms but also their high propensity for unwanted extrapyramidal side-effects and tardive dyskinesia limiting therapeutic benefit. Second generation atypical antipsychotics were developed in an attempt to improve therapy but still have limited effect on cognitive deficits or negative symptoms and many cause weight gain and metabolic abnormalities (Miyamoto et al., 2012). Thus new therapeutic agents operating through different pharmacological mechanisms are essential for more effective management of schizophrenia. One potential mechanism, preferential targeting of the dopamine D_3 receptor over the dopamine D_2 receptor, might provide effective relief of positive and negative symptoms combined with improved cognitive function (Gyertyán et al., 2008; Laszy et al., 2005; Millan and Brocco, 2008; Millan et al., 2008).

Current antipsychotics lack selective discrimination of dopamine D_2 and D_3 receptors, however recent positron emission tomography studies (Howes et al., 2015) show these have a differential brain distribution. The D_3 receptor is abundant in the ventral striatal mesolimbic system and frontal cortex but (unlike the D_2 receptor) low in the dorsal striatum, which may account for the paucity of effect of D_3 antagonists on locomotor activity in rodents and provides potential for few extrapyramidal symptom in clinical use in man (see reviews; Heidbreder and Newman (2010) and Millan and Brocco (2008)). Both gene polymorphisms and increased post-mortem brain D_3 receptor protein levels have also been associated with patients who had schizophrenia. Furthermore converging evidence showing the receptors have contrasting roles on cognition in rodents, encouraged development of drugs with differential D_2/D_3 receptor pharmacology (Millan and Brocco, 2008; Sokoloff et al., 2006; Watson et al., 2012a). Cariprazine (RGH-188; N′-[trans-4-[2-[4-(2,3-dichlorophenyl)-1-piperazinyl]ethyl] cyclohexyl]-N,Ndimethylurea, VraylarTM) is one such orally active, putative antipsychotic which is described as a dopamine D_3 receptor preferring D_3/D_2 receptor partial agonist (Ágai-Csongor et al., 2012; Caccia et al., 2013). *In vitro* ligand binding and functional studies show that cariprazine has 6–10 fold higher affinity for the human dopamine D_3 than D_2 receptor (Ki=0.085 and Ki=0.49 nM, respectively) and is a moderate affinity partial agonist at 5-HT_{1A} receptors (Kiss et al., 2010). Positron emission tomography studies in the monkey confirmed that at the highest dose used (300 μg/kg) cariprazine has >90% D_2/D_3 receptor occupancy in the striatum with only 30% 5-HT_{1A} receptor occupancy in the raphé consistent with the *in vitro* data (Seneca et al., 2011). Unlike other atypical antipsychotics, cariprazine shows a high and balanced occupancy of both D_2 and D_3 receptors in rodents as seen in schizophrenia patients (Kiss et al., 2012; Slifstein et al., 2013). This unique pharmacological profile suggests cariprazine may have antipsychotic effects combined with beneficial effects on cognitive and negative symptoms above that seen with current antipsychotics. Indeed, cariprazine is active in several rodent models predictive of 'antipsychotic-like' activity; including the conditioned avoidance response and inhibition of amphetamine-induced hypermotility, without evidence of induction of 'cataleptic-like' activity (Gyertyán et al., 2011). Furthermore cariprazine has been shown to attenuate the chronic mild stress-induced decrease in sucrose intake in rats which has been suggested by some groups to be a model of anhedonia; a common symptom of schizophrenia as well as depression (Papp et al., 2014). Positive results have been obtained from phase II and III clinical trials in schizophrenia and bipolar mania (Calabrese et al., 2015; Durgam et al., 2014, 2015; Grunder, 2010; Sachs et al., 2015). Recently cariprazine was also shown to produce significant improvement against placebo on Positive and Negative Syndrome Scale total in a 6 week phase III clinical trial (Kane et al., 2015) in patients with an acute exacerbation of schizophrenia and has now received approval by the FDA for the treatment of schizophrenia and acute treatment of manic or mixed episodes associated with bipolar I disorder (McCormack, 2015).

However the preclinical activity of cariprazine against cognitive deficits has so far been limited to reversal of pharmacological impairments in learning and memory. These include scopolamine-induced deficits in a rat water-labyrinth task (where a bell shaped dose–response pattern was observed; Gyertyán et al. (2011)) and PCP-induced deficits in rat and mouse tests of visual recognition memory, attentional set shifting/reversal learning and social interaction/social recognition memory (Adham et al., 2012; Zimnisky et al., 2013).

Rearing rats in social isolation from weaning produces long-term neurodevelopmental, behavioural, structural and neurochemical alterations with translational relevance to a spectrum of changes seen schizophrenia (Fone and Porkess, 2008; Jones et al., 2011b; Lapiz et al., 2003) including visual learning, social cognition, reversal learning and attentional set shifting deficits (Bianchi et al., 2006; Fone et al., 1996; Fone and Porkess, 2008; King et al., 2009; Marsden et al., 2011; Meffre et al., 2012). Importantly in studies examining new potential therapies for schizophrenia compounds with divergent pharmacology including dopamine D_3 or 5-HT_6 receptor antagonists and $mGluR_{2/3}$ or nicotinic receptor agonists (Jones et al., 2011a; King et al., 2013; Marsden et al., 2011; Watson et al., 2012b) reverse components of the isolation-induced syndrome, suggesting this model has useful pharmacological sensitivity. Neonatal treatment of rats with the non-competitive NMDA receptor antagonist, phencyclidine (PCP), disrupts neuronal development and synaptogenesis and also reproduces in adulthood many of the behavioural features akin to schizophrenia, including hyperactivity to amphetamine, attenuated sensorimotor gating and deficits in cognition (Bubenikova-Valesova et al., 2008; Ingallinesi et al., 2015; Mouri et al., 2007). We recently showed that combining neonatal PCP administration with subsequent isolation rearing, as a novel 'dual-hit'