Efficacy of the atypical antipsychotic aripiprazole in \(d\)-amphetamine-based preclinical models of mania

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Abstract

The atypical antipsychotic aripiprazole has been demonstrated to reduce symptoms of bipolar mania. To further profile the antimanic-like properties of aripiprazole in relevant preclinical models, we examined its efficacy in \(d\)-amphetamine-based behavioural models of acute mania in rats. The effects of acute and repeated administration of aripiprazole were assessed in the facilitation of intracranial self-stimulation (ICSS) and hyperlocomotion after acute \(d\)-amphetamine, and in the sensitized facilitation of ICSS function and hyperlocomotion after repeated \(d\)-amphetamine. Acutely, aripiprazole (0.75, 1.5 and 2.5 mg/kg i.p.) increased ICSS thresholds, attenuated the reward-facilitating effects of \(d\)-amphetamine (0.5 mg/kg i.p.), decreased motor activity and prevented \(d\)-amphetamine-induced hyperlocomotion. Co-administration of aripiprazole and \(d\)-amphetamine for 7 d resulted in aripiprazole counteracting the \(d\)-amphetamine-induced sensitization in facilitation of brain reward function and hyperlocomotion. These results indicate the efficacy of aripiprazole in \(d\)-amphetamine-based preclinical models of acute mania that are characterized by increased motivational drive and/or hyperfunction of brain reward.

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Introduction

Bipolar disorder is a debilitating psychiatric illness characterized by changes in mood, which often increase in severity and frequency over time (Belmaker, 2004). Although there have been substantial gains in the treatment of bipolar patients with lithium, anticonvulsants and atypical antipsychotic drugs, many patients fail to respond adequately, or to tolerate these treatments. Importantly, while a specific treatment may be effective for the management of a specific cluster of symptoms, it may not be effective for the management of other clusters. Therefore, the potential value of using animal behavioural models in order to explore the individualized therapeutic action of established mood-stabilizing agents, or to test novel mood stabilizers is unquestionably substantial. However, although attempts have been made to develop a comprehensive model of bipolar disorder, present models do not cover the whole spectrum and the chronic nature of the disorder, mimicking rather its unipolar, cycling phases. For this reason, the majority of studies today use separate models for depression and mania. While a number of valid models are available for the study of the depression pole of bipolar disorder, models for mania are rather sparse.

The clinical hallmark in diagnosing bipolar disorder is the presence of manic symptoms (Belmaker, 2004). Thus, an adequate animal model of the manic pole of bipolar disorder should reflect some features of the acute manic episode, such as hyperactivity, euphoria, increased hedonistic drive and motivation. An animal model that may be particularly useful in the study of motivational states is the intracranial self-stimulation (ICSS) paradigm. This procedure has been used to
evaluate hedonistic properties in rodents and to characterize the motivational effects of drugs. Thus, ICSS behaviour may represent a useful index of altered brain reward function with which to study pre-clinically the acute characteristics of mania. Another behavioural model of acute mania has focused on the hyperactivity aspect of the disorder, as several cardinal symptoms of the manic episode resemble those reported by normal volunteers after d-amphetamine (Amph; Angrist et al. 1987; Jacobs & Silverstone, 1986). Thus, it has been suggested that Amph-induced responses in experimental animals could serve as a model of bipolar mania.

Aripiprazole (Ari) is a second-generation antipsychotic, which has been demonstrated to reduce symptoms of schizophrenia and bipolar mania. Ari has a mechanism of action which differs from all currently marketed atypical antipsychotics (Shapiro et al. 2003). While having some affinity for serotonin 5-HT$_{1A}$ receptors with activity as a partial agonist as well as affinity for 5-HT$_{1A}$ receptors acting as an antagonist, Ari's primary mechanism of action involves both postsynaptic dopamine D$_2$/D$_3$ receptors and presynaptic dopamine autoreceptors. Furthermore, several studies have demonstrated the relatively selective and unique D$_2$ partial agonist properties of Ari.

In the present study, we examined whether Ari affects ICSS and spontaneous motor activity, attenuates the reward-facilitating and motor-stimulating effect of Amph and prevents the sensitization in facilitation of brain reward function and hyperlocomotion induced by repeated Amph.

Methods

Animals

Male Sprague–Dawley rats ($n=64$) weighing 300–350 g were used. Animals were housed 2–3 per cage under a 12-h light/dark cycle (lights on 08:00 hours) with free access to food and water. Experiments were conducted in accordance with the National Institutes of Health ‘Guide for the Care and Use of Laboratory Animals’.

Surgery for ICSS

The animals ($n=32$) were anaesthetized with intramuscular (i.m.) injection of ketamine hydrochloride (100 mg/kg) and xylazine (10 mg/kg). Atropine sulfate (0.6 mg/kg i.m.) was injected to reduce bronchial secretion. The animals were implanted with a monopolar stimulation electrode aimed at the medial forebrain bundle at the level of lateral hypothalamus (2.56 mm posterior to bregma, 1.8 mm lateral from midsagittal suture, and 8.6 below the outer flat skull), according to Paxinos & Watson (2007). The electrodes were constructed from 0.25-mm stainless-steel wire insulated with Epoxylite except for the conically shaped tip. The anode was an uninsulated stainless-steel wire connected to an amphenol pin. Five miniature skull screws, the electrode and the anode were secured to the skull with acrylic dental cement. Following implantation and for the entire duration of the experiments, the animals were housed individually. Stimulation sites were histologically confirmed.

Drugs

Aripiprazole (Otsuka Pharmaceutical Europe Ltd, UK) was diluted in water, and d-amphetamine (Sigma-Aldrich, USA) was dissolved in 0.9% NaCl. Both were injected intraperitoneally (i.p.) at a volume of 1 ml/kg of body weight.

 Procedures for ICSS

After 1 wk recovery, the rats were tested for self-stimulation in an operant chamber made of transparent Plexiglas (25 cm wide, 25 cm deep, 30 cm high). Each chamber was equipped with a stainless-steel poke device (lever) 4 cm wide which protruded 2 cm from the left side at a height of 4 cm from the bottom. Each lever-press triggered a constant current stimulator (Med Associates, USA) that delivered a 0.4-s train of rectangular cathodal pulses of constant duration (0.1 ms) and intensity (250 mA) and variable frequency (15–100 Hz, i.e. 6–40 number of pulses/0.4 s). During the acquisition phase, the animals were trained to self-stimulate for at least three consecutive days (1 h daily), using stimulation parameters that maintained near maximal lever-pressing rates. After self-stimulation had been acquired and stabilized for a given pulse frequency, rats were trained using four alternating series of ascending and descending pulse frequencies. The pulse frequency was varied by steps of $0.1 \log$ units. Each frequency was tested within trials of 60 s duration, followed by an extinction period of 30 s. For each trial, there was an initial ‘priming’ phase during which time the animals received three trains of stimulation at the frequency which was available for the specific trial. A rate-frequency determination session lasted about 45 min. One rate-frequency curve was established daily, for 10–14 d, depending on the period when the self-stimulation indices (i.e. curve shift and threshold measure) were stable. The stimulation parameters, ICSS sessions and data collection were controlled by a computer.
Since both Ari and Amph appear to affect performance capacity in a dose-dependent manner, the use of a reward selective measure, like the curve shift, was requisite. By this method, plotting the responses of the animals against the various pulse frequencies yielded a sigmoidal rate-frequency curve as shown in Fig. 1d. Shifts in the lateral position of the curve provide a selective measure of stimulation-produced reward, while vertical shifts provide information on motor/ performance capacity. Furthermore, this method offers quantitative scaling of drug-induced changes in reward that is useful when comparing the effects of different drugs.

Rats \((n=8)\) received various doses of Ari \((0.075, 0.25, 0.75, 1.5, 2.5 \text{ mg/kg})\) or vehicle in a randomized order. The first session began 15 min post-injection, while the second session started 120 min after Ari administration. Another group of rats \((n=8)\) received Ari \((0.075, 1.5 \text{ mg/kg})\) or vehicle + Amph \((0.5 \text{ mg/kg})\) or vehicle 15 min later; 5 min later the rats were placed in the operant chamber for the post-drug session. In a separate group \((n=16)\), baseline was established (mean of thresholds of a 5-d period) before testing. For the consequent 7-d period, rats received Ari \((1.5 \text{ mg/kg})\) or vehicle + Amph \((0.5 \text{ mg/kg})\) 15 min later.

**Motor activity**

Spontaneous motor activity was measured using an automated recording system (model 7445, Ugo Basile, Italy). Three days before testing, rats were habituated for 30 min daily in the testing chambers. The rats were accustomed to the experimental room, for 1 h prior to the experiment. Rats \((n=12)\) received Ari \((0.075, 1.5 \text{ mg/kg})\) or vehicle + Amph \((0.5 \text{ mg/kg})\) or vehicle 15 min later in a randomized order. For 7 d another group of rats \((n=20)\) received Ari \((1.5 \text{ mg/kg})\) or vehicle + Amph \((0.5 \text{ mg/kg})\) 15 min later.

**Data analysis and statistics**

In the ICSS experiments, analysis was performed on the ICSS threshold as previously described (Mavrikaki et al. 2009). In the acute ICSS experiments, the post-treatment threshold was expressed as percentage of pretreatment values, while in the sensitization experiments it was expressed as percentage of baseline values. In the motor activity experiments, total locomotion and rearing counts over the 30-min observation period were evaluated. The data were statistically analysed using two-way analysis of variance with repeated measures (rm-ANOVA) followed, as appropriate, by rm-ANOVAs and correlated \(t\) tests using Bonferroni’s adjustment for multiple comparisons. When the interaction was significant, we considered Bonferroni’s inequality approach to test the simple effects.

**Results**

**ICSS studies**

**Acute Ari.** Two-way rm-ANOVA demonstrated significant drug \([F(5,35)=22.294, p<0.001]\) and time post-injection effects \([F(1,7)=17.594, p<0.01]\), but no significant interaction. Ari significantly increased self-stimulation thresholds at 0.75–2.5 \text{ mg/kg} \((p<0.05–0.001)\) (Fig. 1).

**Acute Ari + Amph.** Two-way rm-ANOVA showed a significant interaction of Ari + Amph \([F(2,14)=6.884, p<0.01]\). Amph elicited a significant decrease in ICSS threshold \([F(1,7)=57.237, p<0.001]\), and acute Ari \((1.5 \text{ mg/kg})\) blocked Amph’s reward-facilitating effect \([F(2,14)=52.762, p<0.001]\) (Fig. 1).

**Ari–Amph sensitization.** Two-way rm-ANOVA showed a statistical significant time \times treatment interaction \([F(6,84)=128.523, p<0.05]\), and rm-ANOVA on the vehicle + Amph group revealed a significant time effect \([F(6,42)=7.157, p<0.001]\). Post-hoc testing indicated a significant difference between days 1 and 7. rm-ANOVA on the Ari + Amph group revealed no significant time effect. Comparisons between groups demonstrated significant differences (for each day, \(p<0.001\)) (Fig. 1).

**Locomotion studies**

**Ari + Amph.** Two-way rm-ANOVA showed a significant interaction of Ari + Amph \([F(2,22)=15.542, p<0.001]\). Ari affected locomotion \([F(1,11)=23.842, p<0.001]\); acute Ari \((1.5 \text{ mg/kg})\) decreased locomotion \((p<0.001)\), while Ari \((0.075 \text{ mg/kg})\) had no effect. Amph increased locomotion \([F(1,11)=31.918, p<0.001]\). Both acute Ari doses \((p<0.01, p<0.001)\) blocked the Amph-induced hyperlocomotion. Two-way rm-ANOVA on locomotion showed a significant interaction of Ari + Amph \([F(2,22)=9.744, p<0.001]\). Ari affected rearing \([F(1,11)=36.176, p<0.001]\); acute Ari \((1.5 \text{ mg/kg})\) decreased rearing \((p<0.01)\), while Ari \((0.075 \text{ mg/kg})\) had no effect. Amph significantly increased rearing \([F(1,11)=12.031, p=0.005]\). Both acute Ari doses \((p<0.05)\) blocked Amph-increased rearing (Fig. 2).

**Ari–Amph sensitization.** Two-way rm-ANOVA on locomotion showed a statistically significant time \times treatment interaction \([F(6,108)=3.752, p=0.002]\).
Fig. 1. (a) Changes in self-stimulation threshold following acute aripiprazole (Ari; 0, 0.075, 0.25, 0.75, 1.5, 2.5 mg/kg i.p.) or vehicle. Label ‘1’ on the x-axis indicates the first session 15 min after injection of Ari while label ‘2’ indicates the second session 120 min post-injection. Vertical bars represent the means ± S.E. Asterisks signify an ICSS threshold value significantly different...
rm-ANOVA on the vehicle + Amph group revealed a significant time effect \[F(6, 54) = 4.880, p < 0.001\]; only the difference between days 1 and 7 reached statistical significance (\(p < 0.05\)). Comparisons between groups demonstrated significant differences (for each day, \(p < 0.001\)). Two-way rm-ANOVA on rearing showed a significant treatment effect \[F(1, 18) = 34.944, p < 0.001\], but neither significant time nor interaction effects.

**Discussion**

Acute administration of low doses of Ari did not affect the reinforcing efficacy of brain stimulation, whereas higher doses increased ICSS thresholds. The observed effects of Ari on ICSS threshold were relatively long-lasting, since ICSS thresholds remained elevated for 2 h post-injection. This finding indicates that acute administration of Ari reduces brain reward function, producing an anhedonic state. This is contrary to the elation, euphoria and increased hedonistic drive that bipolar patients experience. The effects of Ari on ICSS thresholds are in agreement with previous reports, examining the effects of mood stabilizers (Flagstad et al. 2006; Mavrikaki et al. 2009; Tomasiewicz et al. 2006).

Ari also attenuates Amph’s reward-facilitating effects in ICSS. Our data are consistent with previous from the control group: *\(p < 0.05\), **\(p < 0.01\), ***\(p < 0.001\). (b) Changes in self-stimulation threshold with acute Ari (0.075, 1.5 mg/kg i.p.) or vehicle followed by d-amphetamine (Amph; 0.5 mg/kg i.p.) or vehicle. Vertical bars represent the means ± s.e. Asterisks signify statistically significant different total counts from the control group: **\(p < 0.01\), ***\(p < 0.001\) compared to the control group; +\(p < 0.05\), ++\(p < 0.01\), +++\(p < 0.001\) compared to Amph. (b) Effects of Ari on the development of behavioural sensitization to Amph in motor activity; (i) locomotion, (ii) rearing. Asterisks signify a statistically significant time effect: ***\(p < 0.001\), +++\(p < 0.001\) compared to vehicle + Amph per day. Hash signs signify a statistically significant treatment effect: ###\(p < 0.001\).
studies, showing that Ari counteracts the rewarding/reinforcing effects of psychostimulants in experimental animals and the subjective and discriminative effects of Amph in humans (Feltenstein et al. 2007; Lile et al. 2005; Newton et al. 2008; Sorensen et al. 2008; Stoops et al. 2006; Thomsen et al. 2008; Wee et al. 2007). Ari has been proposed as a potential therapy for psychostimulant dependence (Stoops, 2006) or for the treatment of schizophrenia with comorbid stimulant abuse (Beresford et al. 2005). Interestingly, one of the main symptoms of mania is the propensity towards drug abuse, and bipolar patients have a high rate of co-existing substance use disorder (Altamura, 2007). The applicability of these findings in the treatment of bipolar disorder comorbid with substance abuse remains to be determined in the clinic.

The data presented in Fig. 1b suggest that the ability of Ari to attenuate the effect of Amph on ICSS is related to increases in baseline ICSS threshold produced when Ari is administered alone. In other words, the ability of a particular dose of Ari to attenuate Amph’s effects on ICSS is correlated with the threshold-increasing effect of that dose. Opposing actions of dopamine receptor antagonists and psychostimulants in the ICSS paradigm are not uncommon (see e.g. Gallistel & Karras, 1984). We hypothesize that the dose of Ari needed to block the euphoric effects of psychostimulants, such as Amph, also interferes with the normal functioning of the brain reward system, since the dose needed to attenuate or block the psychostimulant effects increase ICSS thresholds when administered alone. However, it should be noted that an increase in threshold frequency produced by a compound does not always result in a decrease in the reward-facilitating effect of a psychostimulant. For example, in our previous study, although valproic acid increased baseline ICSS threshold it did not attenuate the reward-facilitating effect of Amph (Mavrikaki et al. 2009).

The observation that a relatively low dose of Ari (0.075 mg/kg) tended to increase the reward-facilitating effects of Amph in the ICSS paradigm indicates a rather synergistic action between the two drugs at least in this dose combination. This effect, although not reaching significance in our study, is in agreement with a recent study in which Ari increases some of the rewarding and stimulatory effects produced by acute methamphetamine in humans (Newton et al. 2008). The precise mechanisms through which a low dose of Ari increases the reward-facilitating effects of Amph remain to be established.

Ari inhibited the Amph-induced hyperlocomotion in rats, even at low doses that did not affect motor activity. Psychostimulant-induced hyperactivity has been proposed as a preclinical model for mania-like behaviour and has been shown to be sensitive to drugs used for the treatment of bipolar disorder (Eina! 2006; Einat et al. 2003; Hasler et al. 2006). Our results are in accord with other reports, showing that Ari counteracts the hyperactivity induced by psychostimulants in rodents (Jerlhag, 2008; Leite et al. 2008; Nordquist et al. 2008) and the subject-rated effects of Amph in humans (Lile et al. 2005; Stoops et al. 2006).

Ari inhibited both the sensitized facilitation of brain reward function and the behavioural sensitization in the motor activity induced by repeated Amph. Behavioural sensitization, which is commonly observed following chronic treatment with psychostimulants, has been used to model the development of bipolar disorder (Einat, 2006). Moreover, it has been shown that mood stabilizers prevent the development of behavioural sensitization (Eckermann et al. 2001). The development of sensitization of brain reward function after repeated Amph is the result of drug-induced changes in the reinforcing efficacy of the stimulation, as indicated by the progressive decrease in ICSS threshold. Namely, the progressive lowering of ICSS thresholds observed after repeated Amph reflects an enduring increase in brain reward function that is assumed to underlie its manic-producing effects (see e.g. Strakowski et al. 1996). Sensitization of ICSS and hyperlocomotion after repeated Amph is consistent with previous reports (Predy & Kokkindis, 1984). The present results corroborate the notion that dopamine-mediated behaviours are facilitated after prolonged drug treatment. Indeed, the same dopamine pathways that play an important role in drug dependence and psychosis also play a critical role in behavioural sensitization. A plausible explanation for these effects may be the reported decreased sensitivity of somatodendritic D2 autoreceptors following repeated Amph (White & Wang, 1984). Thus, Ari may act to normalize dopaminergic neurotransmission and prevent the development of behavioural sensitization.

In summary, the present study shows that low doses of acute Ari did not affect the reinforcing efficacy of brain stimulation, whereas higher doses increased ICSS thresholds. Moreover, Ari effectively inhibited the reward-facilitating effects of Amph in the ICSS as well as the hyperlocomotor responses induced by Amph in the open field. Finally, Ari counteracted the sensitized facilitation of brain reward function after repeated Amph and the behavioural sensitization in motor activity induced by Amph. An important consideration of the present study is the relevance of our findings and the sensitivity of the models used to other...
mood-stabilizing agents. Furthermore, emerging clinical evidence suggest that acute manic or mixed episodes of bipolar disorder are responsive to Ari. Therefore, our results suggest that Ari might ameliorate symptoms of conditions characterized by increased motivational drive and/or hyperfunction of brain reward systems, such as mania and psychostimulant abuse and may have therapeutic value for the treatment of euphoric mania or bipolar disorder comorbid with substance abuse. These observations strengthen the position that ICSS and motor activity involving Amphetamine administration constitute useful models to explore the elation, increased hedonistic drive and hyperactivity observed in the acute phase of manic episodes of patients with bipolar disorder and ultimately help to identify novel pharmacotherapies.

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Statement of Interest

None.

References


