Pharmacology, Biochemistry and Behavior 153 (2017) 141–146

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Involvement of presynaptic 5-HT$_{1A}$ receptors in the low propensity of brexpiprazole to induce extrapyramidal side effects in rats

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ARTICLE INFO

Article history:
Received 8 July 2016
Received in revised form 3 November 2016
Accepted 31 December 2016
Available online 3 January 2017

Keywords:
Catalepsy
Microdialysis
Brexpiprazole
Aripiprazole
5-HT$_{1A}$ receptor agonists
D$_2$ receptor agonists

ABSTRACT

Previous studies have shown that partial and full 5-HT$_{1A}$ receptor agonists reduce antipsychotic-induced catalepsy. Consequently, some antipsychotics combining balanced efficacy between dopamine (DA) D$_2$ antagonism or partial agonism and 5-HT$_{1A}$ receptor agonism have a low propensity to induce extrapyramidal side effects (EPS), as reflected by low cataleptogenic activity in rodents. In the present experiments, we attempted to explore the importance of pre- and postsynaptic 5-HT$_{1A}$ agonistic properties of brexpiprazole and aripiprazole in the context of neurological side-effect liabilities. Additional measures of prefrontal cortical serotonin (5-HT) and DA levels using microdialysis were used to support that brexpiprazole has a preferential agonist effect on presynaptic 5-HT$_{1A}$ receptors.

Brexpiprazole (3.0 and 10 mg/kg, p.o.) as well as aripiprazole (8.0 and 30 mg/kg, p.o.) failed to induce catalepsy in rats. Brexpiprazole (10 mg/kg, p.o.) significantly reduced the cataleptic response induced by haloperidol (0.63 mg/kg, s.c.), while aripiprazole (1.0–100 mg/kg, p.o.) failed to reverse the effect of haloperidol and only showed a numeric decrease at 10 mg/kg, (p.o.).

When 5-HT$_{1A}$ receptors were blocked by the selective antagonist, WAY100635 (1.0 mg/kg, s.c.), cataleptogenic properties of brexpiprazole (10 mg/kg; p.o) but not aripiprazole (8.0 and 30 mg/kg, p.o.) were unmasked.

The ("biased") 5-HT$_{1A}$ receptor agonists F15599 (postsynaptic preference) and F13714 (presynaptic preference) had differential effects on haloperidol-induced catalepsy: F13714 (0.16 mg/kg, s.c.) counteracted catalepsy, whereas F15599 (0.040 mg/kg, s.c.) had no significant effect at regionally-selective doses. These data support a role of presynaptic 5-HT$_{1A}$ receptors in the anticataleptic effect of brexpiprazole. The selective 5-HT$_{2A}$ antagonist M100907 (0.10 mg/kg, s.c.) had no effect on haloperidol-induced catalepsy, arguing against a major role of 5-HT$_{2A}$ receptors in the cataleptogenic profile of brexpiprazole.

The findings with brexpiprazole were supported using microdialysis studies: Brexpiprazole (3.0 and 10 mg/kg, p.o.) decreased extracellular 5-HT levels in the medial prefrontal cortex (mPFC), while it failed to affect extracellular DA in the same samples, suggesting that the 5-HT$_{1A}$ agonist properties of brexpiprazole may be preferentially presynaptic.

In conclusion, these results confirm that brexpiprazole and aripiprazole have low propensities to induce EPS. However, the low EPS risk of brexpiprazole is more likely dependent on its agonist properties on presynaptic 5-HT$_{1A}$ receptors, while that of aripiprazole is less sensitive to 5-HT$_{1A}$ receptor antagonism.

1. Introduction

Brexpiprazole and aripiprazole are partial dopamine (DA) D$_2$ agonists approved in the United States for treatment of schizophrenia and as adjunctive treatment of major depressive disorder. In clinical trials both drugs have shown low potential for occurrence of extrapyramidal side effects (EPS; Correll et al., 2015; Kane et al., 2015, Thase et al., 2015a, 2015b; Gao et al., 2008; Rummel-Kluge et al., 2012), although a recent explorative study comparing both drugs in patients with schizophrenia report a lower incidence of akathisia in patients treated with brexpiprazole compared to those treated with aripiprazole (Citrome et al., 2016). However, it is unclear whether this low EPS liability is due to their partial DA agonist activity as compared to DA D$_2$ antagonist antipsychotics, or if it is mainly driven by their additional partial agonist activity at 5-HT$_{1A}$ receptors (Bardin et al., 2007; Newman-Tancredi and Kleven, 2011). Consistent with clinical experience, brexpiprazole

Abbreviations: ANOVA, Analysis of variance; DA, Dopamine; EPS, Extrapyramidal side effects; L-DOPA, L-3,4-dihydroxyphenylalanine; mPFC, Medial prefrontal cortex; 5-HT, Serotonin.

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http://dx.doi.org/10.1016/j.pbb.2016.12.015
0091-3057/

and aripiprazole do not or only weakly induce catalepsy in rodents (Bardin et al., 2007; Maeda et al., 2014b), the most commonly used predictive animal test for parkinsonian-like EPS in humans (Arnt, 1982; Arnt and Skarsfeldt, 1998).

Furthermore, it is not known whether the low EPS potential of brexpiprazole and aripiprazole is a result of similar mechanisms of action, as their pharmacological profiles show some distinct differences. For instance, brexpiprazole has lower D_2 relative agonist efficacy, higher relative affinity for 5-HT_{1A} receptors (Maeda et al., 2014a) and more balanced antagonist effects on 5-HT_{2A} receptors (Oosterhof et al., 2014) as compared to aripiprazole. The majority of second generation D_2 antagonist antipsychotics have potent 5-HT_{2A} antagonist activity, which may also contribute to their lower EPS potential (Arnt and Skarsfeldt, 1998).

It is well-known that 5-HT_{1A} receptor agonists, and in particular high-efficacy agonists, reverse catalepsy induced by the classical D_2 antagonist antipsychotic haloperidol in rodents (Invernizzi et al., 1988; Prinssen et al., 2002). More recently, "biased" 5-HT_{1A} agonists with functional preference for presynaptic autoreceptors in dorsal raphe nucleus versus postsynaptic 5-HT_{1A} heteroreceptors in medial prefrontal cortex (mPFC) have been developed (Newman-Tancredi et al., 2009; Assié et al., 2010; Lladó-Pelfort et al., 2010), and it was observed that agonists with preferential autoreceptor effects are more effective than postsynaptic agonists in reversing symptomatology in rat models of Parkinsonism (e.g. L-DOPA-induced abnormal involuntary movements (Iderberg et al., 2015).

The present study compared the influence of brexpiprazole, aripiprazole, the two biased 5-HT_{1A} agonists, F15599 and F13714 (Iderberg et al., 2015) and a selective 5-HT_{2A} antagonist, M100907 (Sorensen et al., 1993), on haloperidol-induced catalepsy in rats. Additionally, the effects of brexpiprazole and aripiprazole were studied when given alone and after co-treatment with the selective 5-HT_{1A} antagonist, WAY100635 (Forster et al., 1995). Lastly, the effect of brexpiprazole on extracellular levels of 5-HT and DA in the mPFC was explored using microdialysis, since the regulation of the levels of these two transmitters by 5-HT_{1A} agonists is suggested to reflect mainly stimulation of pre- and postsynaptic 5-HT_{1A} receptors, respectively (Lladó-Pelfort et al., 2010, 2012; Iderberg et al., 2015).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles River, Crl:CD Germany) weighing 175–200 g at the time of testing (catalepsy experiments) or 225–250 g (microdialysis experiments) were used. The rats arrived from the breeder at least 5 days before being used in experiments and were housed 4 per cage in Macrolon type III cages and maintained on a 12 h light/dark cycle (lights on 06:00). Water and food was available ad libitum. Temperature (21 ± 2 °C) and relative humidity (55 ± 5%) were automatically controlled.

All experimental protocols were approved by Lundbeck Animal Ethical Committee. Conditions related to the animals used and the in-life experimental procedures undertaken during the course of the study are consistent with the Danish Executive Orders on Animal Testing No. 88 of 30 January 2013 and No. 253 of 08 March 2013.

2.2. Catalepsy

Catalepsy was evaluated using a vertical metal grid (50 × 50 cm^2), with mesh openings of 1 × 1 cm^2) on to which individual rats were gently placed with their heads pointing toward the ceiling and all paws gripping the grid. Latency to move both forepaws to relocate the body was measured with a maximum cutoff time of 30 s. The median latency time over three trials was calculated and used as catalepsy score for each rat. Kruskal-Wallis One Way Analysis of Variance on Ranks followed by appropriate All Pairwise Multiple Comparison Procedures (Dunn's Method) was used to investigate statistical differences between test groups ($P < 0.05$). The non-parametric test was selected because the distribution of catalepsy latencies deviated from normal distribution, because of the 30 s cut-off. Numbers of rats in each experimental group are indicated in the respective figure legends.

2.2.1. Experiment 1

Rats received brexpiprazole (1.0; 3.0; 10; 30 mg/kg, p.o), aripiprazole (1.0; 10; 30; 100 mg/kg, p.o.) or 10% HP-β-cyclodextrin vehicle (5 ml/kg, p.o.). Ninety minutes later the rats received haloperidol (0.63 mg/kg, s.c.). Catalepsy score was assessed 30 min after haloperidol administration.

2.2.2. Experiment 2

Rats received brexpiprazole (3.0; 10 mg/kg p.o), aripiprazole (8.0; 30 mg/kg, p.o.) or 10% HP-β-cyclodextrin vehicle (5 ml/kg, p.o.). Ninety minutes later, rats were injected with WAY100635 (1.0 mg/kg, s.c.) or saline (5 ml/kg, s.c.). Catalepsy score was assessed 30 min after WAY100635 administration.

2.2.3. Experiment 3

Catalepsy score was measured 30 min after M100907 (0.10 mg/kg, s c) alone or in combination with haloperidol (0.63 mg/kg, s.c) or saline (5 ml/kg, s.c.).

2.2.4. Experiment 4

Rats received F15599 (0.010; 0.020; 0.040 mg/kg, s.c.), F13714 (0.010; 0.040; 0.16 mg/kg, s.c.) or 5% HP-β-cyclodextrin vehicle (5 ml/kg, s.c.). Thirty minutes later, rats received haloperidol (0.63 mg/kg, s.c.) or saline (5 ml/kg, s.c.). Catalepsy score was assessed 30 min after haloperidol administration. Doses of the biased 5-HT_{1A} agonists were selected to ensure optimum selectivity for post- and presynaptic receptors, respectively. Particularly for the postsynaptic agonist, F15599, it has been shown that at doses of 0.16 mg/kg and higher the postsynaptic selectivity is lost (Lladó-Pelfort et al., 2010; Iderberg et al., 2015).

2.3. Microdialysis studies

Rats were anaesthetised with Hypnorm/Dormicum (2 ml/kg), and intracerebral guide cannulas (CMA/12) were stereotaxically implanted into the brain, aiming to position the dialysis probe tip in the medial prefrontal cortex (mPFC); co-ordinates: 3.2 mm anterior to bregma, lateral, 0.8 mm, 4.0 mm ventral to dura, according to Paxinos and Watson (1986). Anchor screws and acrylic cement were used for fixation of the guide cannulas. The body temperature of the animals was monitored by rectal probe and maintained at 37 °C. The rats were allowed to recover from surgery for 2 days, housed singly in cages.

On the day of the experiment a microdialysis probe (CMA/12, 0.5 mm diameter, 3 mm length) was inserted through the guide cannula. The probes were connected via a dual channel swivel to a microinjection pump. Perfusion of the microdialysis probe with filtered Ringer solution (145 mm NaCl, 3 mM KCl, 1 mM $MgCl_2$, 1.2 mM $CaCl_2$ containing 0.5 μM escitalopram) begun shortly before insertion of the probe into the brain and continued for the duration of the experiment at a constant flow rate of 1 μl/min. After 180 min of stabilization, dialysates were collected every 20 min. A total of 12 fractions were sampled. Following collection of 4 basal samples, brexpiprazole (1.0; 3.0; 10 mg/kg, p.o.) was administered. The dialysate was analysed using HPLC with electrochemical detection. After the experiments the animals were sacrificed and the brains removed and the probe placement was verified.

Analysis of dialysate for 5-HT and DA: The content of 5-HT and DA in the dialysates was analysed by means of HPLC with electrochemical detection. The monoamines were separated by reverse phase liquid