Progress in Neuro-Psychopharmacology & Biological Psychiatry 36 (2012) 60–70

Contents lists available at SciVerse ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

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

Increasing the density of the D_{2L} receptor and manipulating the receptor environment are required to evidence the partial agonist properties of aripiprazole

Beryl Koener, Marylène C. Focant, Barbara Bosier, Jean-Marie Maloteaux, Emmanuel Hermans *

Institute of Neuroscience (Ions), Group of Neuropharmacology, Université Catholique de Louvain, Avenue Mounier 53, bte B1.53.02, B-1200 Brussels, Belgium

ARTICLE INFO

Article history:
Received 17 June 2011
Received in revised form 5 August 2011
Accepted 10 August 2011
Available online 17 August 2011

Keywords:
(−)-3-PPP
G protein
Inducible expression
N-methyl-D-glucamine

ABSTRACT

The clinical efficacy of aripiprazole in the treatment of psychosis relies on a partial agonism at D_2 receptors. As the expression of this receptor differs physiologically between pre- and post-synaptic sites and is affected by pathological conditions or pharmacological treatments, it appears difficult to predict the clinical response to partial agonists. In addition, the response to this novel antipsychotic was shown to depend on the cell-line and the pathway analyzed, suggesting a functional selective profile at the D_2 receptor. This study aims at examining the influence of receptor density and ionic environment on the pharmacological properties of aripiprazole. A cell line was developed in which the expression of the recombinant D_2 receptor can be tightly manipulated using doxycycline and sodium butyrate. The potency and efficacy of aripiprazole and other reference D_2 receptor ligands were examined in [^{35}S]GTPγS binding assays, in buffers containing either NaCl or N-methyl-D-glucamine (NMDG) which is proposed to enhance G protein coupling. Increasing the density of D_2 receptors considerably enhanced the [^{35}S]GTPγS binding induced by dopamine and the full agonist NPA. In maximally induced cells, the agonist properties of the partial agonist (−)-3-PPP was revealed in a buffer containing NaCl, whereas the response to aripiprazole was not evidenced. Substituting NMDG for NaCl promoted the response to dopamine and (−)3-PPP and was proven efficient to reveal the partial agonist profile of aripiprazole. While NMDG substitution for NaCl strongly enhanced receptor-G protein coupling, these ionic manipulations are likely to influence receptor conformations, thereby modulating the activation of signaling pathways. Our data obtained with partial agonists acting at the D_2 receptor suggest that these changes in the experimental conditions could contribute to reveal the functional selective profile of GPCR ligands. They also emphasize that the properties of functional selective ligands do not only depend on receptor density but also on the surrounding environment which likely differs between brain structures.

1. Introduction

The inhibition of dopamine transmission, through antagonism at D_2 receptors, is a key aspect in the pharmacological management of psychosis (Creese et al., 1976; Seeman et al., 1976). Side effects resulting from to this blockade can be balanced by either 5-HT_{2A} antagonism but also by a rapid dissociation from the D_2 receptor (Kapur and Seeman, 2001), two properties shared by atypical antipsychotics and that account for their atypical profile. Recently, stabilization of the dopamine transmission by compounds endowed with partial agonism at D_2 receptor was proposed as an alternative to the conventional receptor blockade. Several high affinity partial agonists of the D_2 receptors have been developed (Bardin et al., 2006; Kiss et al., 2010), among which aripiprazole, launched in 2002, is considered as the first member of this new class of antipsychotics (Burris et al., 2002). Nevertheless, even though the fine-tuning of dopamine transmission by these compounds is an attractive hypothesis to explain their clinical efficacy (Tamminga and Carlsson, 2002), the ideal intrinsic activity of these partial agonists remains unclear. Hence, the partial agonism of aripiprazole at dopamine receptors in native brain tissue remains a matter of debate (Jordan et al., 2007; Koener et al., 2011).

It is established that the intrinsic activity of a given partial agonist varies, depending on the density of targeted receptors in the tissue (Hermans et al., 1999; Watts et al., 1995). Accordingly, when focusing on the modulation of second messengers such as the inhibition of cAMP accumulation in cells expressing low levels of the D_2 receptor, aripiprazole and (S)-(−)-3-(3-hydroxyphenyl)-N-propylpiperidine hydrochloride ((−)-3-PPP) behave as antagonists whereas an agonist profile is clearly evidenced in models where the receptor density is high (Burris et al., 2002; Lawler et al., 1999; Tadori et al., 2005, 2009). In *in vivo* studies, aripiprazole was shown to display partial agonism at the presynaptic receptor and antagonism at the postsynaptic site (Kikuchi et al., 1995; Kiss et al., 2010; Momiyama et al., 1996; Semba et al., 1995), where high and low receptor reserves are commonly

* Corresponding author at: Université catholique de Louvain, Institute of Neurosciences, Group of Neuropharmacology, Avenue Mounier 53, bte B1.53.03, 1200 Brussels, Belgium. Tel.: +32 2 7649339; fax: +32 2 7645460.
E-mail address: emmanuel.hermans@uclouvain.be (E. Hermans).

0278-5846/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.pnpbp.2011.08.007

evidenced, respectively (Meller et al., 1986). In addition to the question of receptor density, the partial agonism of aripiprazole at dopamine receptors also depend on the cell line used and the functional response examined (Burris et al., 2002; Jordan et al., 2007; Shapiro et al., 2003; Urban et al., 2007). Thus, when examining the modulation of independent signaling pathways in a given cell type, large differences in the estimated efficacy and potency of aripiprazole were reported, suggesting that this drug is endowed with functional selective properties (Shapiro et al., 2003; Urban et al., 2007). There is indeed accumulating evidence for the multiplicity of couplings of the majority of GPCRs, including dopamine receptors, which can be manipulated by such functional selective ligands (Bosier and Hermans, 2007; Hermans, 2003; Kilts et al., 2002; Mottola et al., 2002). Guanylyl

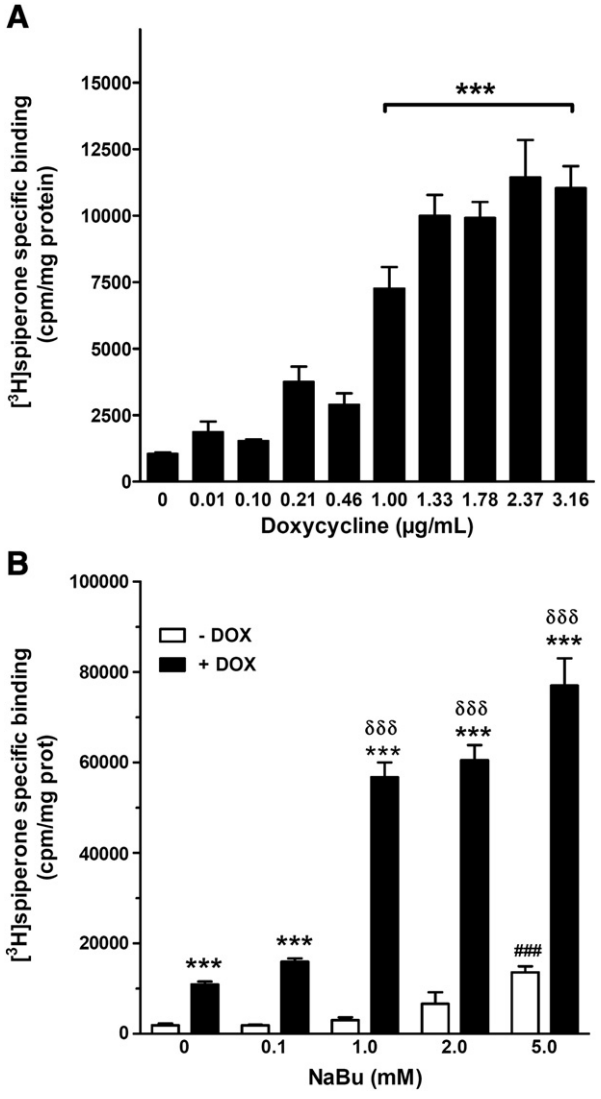

Fig. 1. Inducible expression of D_{2L} receptors. The specific binding of [^{3}H]spiperone was measured on homogenates from Hela-Tet-On D_{2L} cells in NaCl binding buffer. A. [^{3}H] spiperone (1 nM) binding experiments were conducted on cells cultured for 48 h in the presence of increasing concentrations of doxycycline ranging from 0 to 3.16 µg/mL. Shown is a representation of means with s.e.mean from a single experiment repeated three times independently in triplicate. B. The specific binding of [^{3}H]spiperone (2 nM) was determined on cells cultured in the absence (open bars) or in the presence (closed bars) of 2 µg/mL of doxycycline for 48 h, with or without the addition of NaBu (0, 0.1, 1, 2 and 5 mM) during the last 24 h. Data are shown as means with s.e.mean for 3 separate experiments performed in triplicate. ### $p<0.001$ denotes a significant effect associated with the addition of NaBu (comparison with the data obtained without NaBu), *** $p<0.001$ denotes a significant influence of doxycycline for each concentration of NaBu (one-way ANOVA, followed by Dunnett's test for multiple comparisons). **δδδ** $p<0.001$ denotes difference between influence of the combination of doxycycline and NaBu compared to doxycycline alone.

Table 1
Pharmacodynamic parameters derived from [^{3}H]spiperone and [^{35}S]GTPγS binding assays on cells cultured in the presence of increasing concentrations of doxycycline.

Dox (µg/mL)	[^{3}H]spiperone specific binding				Dopamine				NPA			
	B_{max} cpm 10^3/mg prot		K_D (pM)		pEC_{50}		E_{max} (% above basal)		pEC_{50}		E_{max} (% above basal)	
	NaCl	NMDG	NaCl	NMDG	NaCl	NMDG	NaCl	NMDG	NaCl	NMDG	NaCl	NMDG
CTRL	N.D.	N.D.	N.D.	N.D.	4.56 ± 0.39	5.85 ± 0.71	10.81 ± 2.01	7.04 ± 1.82	N.D.	8.43 ± 0.47	N.D.	10.24 ± 1.86
0.01	N.D.	1.6 ± 0.5	N.D.	126.2 ± 79.3	3.94 ± 1.18	6.25 ± 0.11*	7.94 ± 5.73	21.09 ± 0.80	7.23 ± 0.80	8.42 ± 0.18	7.73 ± 3.38	19.03 ± 1.28**
0.10	2.2 ± 0.2	2.7 ± 1.1	212.9 ± 68.2	31.4 ± 7.7*	3.43 ± 0.93	5.98 ± 0.21**	26.17 ± 19.20	24.44 ± 1.87	7.20 ± 2.15	8.73 ± 0.13	14.78 ± 17.49	26.83 ± 1.24
0.21	4.1 ± 0.7	3.5 ± 0.9	156.2 ± 29.7	144.6 ± 62.7	4.34 ± 0.27	6.44 ± 0.12**	28.12 ± 3.96	35.50 ± 7.49	7.93 ± 0.58	8.83 ± 0.17	24.60 ± 6.39	33.92 ± 2.10
0.46	4.1 ± 0.7	3.8 ± 0.6	ND	38.2 ± 4.5	4.64 ± 0.23	5.94 ± 0.16**	46.33 ± 4.99	38.15 ± 2.28	8.22 ± 0.20	8.49 ± 0.23	31.47 ± 2.62	37.56 ± 2.84
1.00	7.7 ± 0.6	7.9 ± 0.1	97.5 ± 32.3	58.8 ± 17.3	4.39 ± 0.20	6.27 ± 0.18***	52.53 ± 5.25	44.57 ± 2.77	8.13 ± 0.90	9.03 ± 0.17	37.70 ± 12.34	48.61 ± 2.95
1.33	11.4 ± 1.3	8.2 ± 0.9*	99.8 ± 27.0	51.7 ± 18.6	4.44 ± 0.26	6.30 ± 0.05***	59.42 ± 7.61	55.61 ± 1.01	8.18 ± 0.19	8.84 ± 0.08**	41.57 ± 3.33	57.14 ± 1.64**
1.78	11.7 ± 0.6	10.7 ± 1.9	96.2 ± 10.6	57.5 ± 7.4**	4.80 ± 0.16	6.10 ± 0.09***	60.41 ± 4.43	53.38 ± 1.63	8.28 ± 0.13	8.88 ± 0.13**	56.06 ± 2.94	52.40 ± 2.58
2.37	13.1 ± 0.2	12.1 ± 1.2	94.7 ± 26.1	55.7 ± 10.3	4.71 ± 0.16	6.15 ± 0.09***	71.18 ± 5.32	55.98 ± 1.72**	8.24 ± 0.16	8.91 ± 0.16**	55.86 ± 3.61	65.04 ± 3.72*
3.16	12.1 ± 0.1	12.9 ± 1.6	92.1 ± .6	52.7 ± 6.4*	4.98 ± 0.12	6.18 ± 0.10***	77.21 ± 3.86	59.29 ± 2.01**	8.21 ± 0.09	8.97 ± 0.11***	61.67 ± 2.26	58.32 ± 2.40

Shown are B_{max} and K_D values from the binding saturation-curves performed in NaCl and NMDG containing buffer. pEC_{50} values and E_{max} values were derived from the dopamine- and NPA-induced [^{35}S]GTPγS binding. Data are expressed as means ± s.e.mean for 3 independent experiments, each performed in triplicate. At single concentrations of doxycycline, parameters measured in buffers containing NaCl or NMDG were compared using unpaired Student's *t*-test. * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ denote differences between values obtained in the two buffers; N.D.: not determined.