Brain Research 1676 (2017) 19–27

Contents lists available at ScienceDirect

Brain Research

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

Research report

Monoamine involvement in the antidepressant-like effect induced by P2 blockade

Cassiano R.A.F. Diniz [a], Murilo Rodrigues [b], Plínio C. Casarotto [c], Vítor S. Pereira [d], Carlos C. Crestani [e], Sâmia R.L. Joca [b,d,*]

[a] Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Brazil
[b] Department of Physics and Chemistry, School of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Brazil
[c] Neuroscience Center, University of Helsinki, Finland
[d] Department of Clinical Medicine – Translational Neuropsychiatry Unit, Aarhus University, Denmark
[e] Laboratory of Pharmacology, School of Pharmaceutical Sciences, UNESP – Universidade Estadual Paulista, Araraquara, Brazil

ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form 6 September 2017
Accepted 8 September 2017
Available online 12 September 2017

Keywords:
Antidepressant
ATP
Serotonin
Noradrenaline
Forced swimming test

ABSTRACT

Depression is a common mental disorder that affects millions of individuals worldwide. Available monoaminergic antidepressants are far from ideal since they show delayed onset of action and are ineffective in approximately 40% of patients, thus indicating the need of new and more effective drugs. ATP signaling through P2 receptors seems to play an important role in neuropathological mechanisms involved in depression, since their pharmacological or genetic inactivation induce antidepressant-like effects in the forced swimming test (FST). However, the mechanisms involved in these effects are not completely understood. The present work investigated monoamine involvement in the antidepressant-like effect induced by non-specific P2 receptor antagonist (PPADS) administration. First, the effects of combining sub-effective doses of PPADS with sub-effective doses of fluoxetine (FLX, selective serotonin reuptake inhibitor) or reboxetine (RBX, selective noradrenaline reuptake inhibitor) were investigated in mice submitted to FST. Significant antidepressant-like effect was observed when subeffective doses of PPADS was combined with subeffective doses of either FLX or RBX, with no significant locomotor changes. Next, the effects of depleting serotonin and noradrenaline levels, by means of PCPA (p-Chlorophenylalanine) or DSP-4 (N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine hydrochloride) pretreatment, respectively, was investigated. Both, PCPA and DSP-4 pretreatment partially attenuated PPADS-induced effects in FST, without inducing relevant locomotor changes. Our results suggest that the antidepressant-like effect of PPADS involves modulation of serotonin and noradrenaline levels in the brain.

1. Introduction

World Health Organization (WHO) estimates 350 million of individuals are affected by depression globally, and it predicts depression will be the main cause of morbidity and loss of productivity among all health conditions by 2030 (World Health Organization, 2016). The current antidepressant drug therapy, based on increasing monoamines availability (Elhwuegi, 2004), is only effective in approximately 60% of the patients, and it takes 3–4 weeks to be clinically effective (Blier, 2003; Racagni and Popoli, 2008).

Great attention has been paid to the purinergic signaling in neuropathological disorders such as Alzheimer and Parkinson disease, anxiety, schizophrenia, drug addiction, and, of importance to the present work, depression, (Abbracchio et al., 2009; Burnstock, 2008). Purinergic neurotransmission emerged from the work of Prof. Burnstock describing adenosine 5'-triphosphate (ATP) as non-adrenergic, non-cholinergic inhibitory transmitter in the guinea-pigs (Abbracchio and Burnstock, 1994; Burnstock, 1972; Burnstock, 1976). Since then, ATP has been described as a cotransmitter in noradrenergic (Sperlagh et al., 1998), gabaergic (Jo and Role, 2002), glutamatergic (Mori et al., 2001) and cholinergic (Richardson and Brown, 1987) synaptic terminals, but vesicles containing exclusively ATP have also been reported (Pankratov et al., 2006). ATP released in synaptic cleft can be degraded by

* Corresponding author at: Department of Physics and Chemistry – School of Pharmaceutical Sciences of Ribeirão Preto (FCFRP), University of São Paulo (USP), AvCafe, s/n, 14040-903 Ribeirão Preto, SP, Brazil.
E-mail addresses: cassianodiniz4@hotmail.com (C.R.A.F. Diniz), murilorodrigues43@gmail.com (M. Rodrigues), plinio@gmx.com (P.C. Casarotto), vitor.silvapereira@gmail.com (V.S. Pereira), cccrestani@yahoo.com.br (C.C. Crestani), samia@usp.br (S.R.L. Joca).

http://dx.doi.org/10.1016/j.brainres.2017.09.011
0006-8993/

ectonucleotidases into various metabolites, such as adenosine, which also acts as a ligand for purinergic receptors (Zimmermann, 2006).

Purinergic neurotransmission comprises receptors for adenosine and ATP, respectively termed P1 and P2. Based on molecular cloning and pharmacological differences, P2 receptors can be divided into P2Y and P2X receptors (Abbracchio and Burnstock, 1994). P2Y are G-protein coupled receptors that modulate inositol-1,4,5-triphosphate (IP3) and diacylglycerol (DAG) levels from membrane phosphoinositide metabolism (Pfeilschifter, 1990; Burnstock, 2007), while P2X are ion channels permeable to Na^+, K^+ and Ca^{2+} (Burnstock, 2007; Benham and Tsien, 1987; Bean, 1992). Currently, 8 subtypes of metabotropic P2Y receptors ($P2Y_{1,2,4,6,11,12,13,14}$) and 7 subtypes of ionotropic P2X receptors ($P2X_{1-7}$) have been described (Burnstock, 2007). P2 receptors are widely expressed in cerebral structures involved in emotional behavior such as hippocampus, cerebral cortex, ventral tegmental area and locus coeruleus (Norenberg and Illes, 2000), either in glial or neuronal cell types (Burnstock, 2008).

It has been recently reported that pharmacological blockade of P2 receptors induces antidepressant-like effects in preclinical models (Pereira et al., 2013; Csolle et al., 2013). Selective P2X7 antagonist also induced antidepressant-like effect in the chronic mild stress model (Iwata et al., 2016) and similar behavioral phenotype was also described in animals with genetic deletion of P2X7 receptors (Boucher et al., 2011; Basso et al., 2009). Despite evidences of an antidepressant-like effect induced by P2R antagonist treatment, the involvement of serotonergic and/or noradrenergic mechanisms has not yet been investigated. Monoamine and purinergic interplay is plausible given the fact that activation of P2R can modulate brain glutamate and NO levels (Florenzano et al., 2008; Pereira et al., 2013), which are both able to control serotonin synthesis, stability and release (Kuhn and Arthur, 1997; Fossier et al., 1999). In support of that, P2X7R knockout animals showed changes in cerebral levels of noradrenaline and serotonin (Csolle et al., 2013). Furthermore, the antidepressant-like effect of NOS inhibitors and NMDA antagonists (Diniz et al., 2016) are dependent of serotonin levels in the brain (Harkin et al., 2003; du Jardin et al., 2016; Ulak et al., 2010). Therefore, since P2R signaling modulate glutamate and NO release in the brain, which then affects monoamine levels, we hypothesized the antidepressant-like effect of the non-specific P2R antagonist PPADS would involve the modulation of brain serotonin and/or noradrenaline levels.

Therefore, the present aims were: 1. To investigate if the combination of sub-effective doses of P2R antagonist with subeffective doses of antidepressants of different pharmacological classes (serotonin or noradrenaline reuptake inhibitors) would be able to induce antidepressant-like effect in mice submitted to forced swimming test; 2. To investigate if depleting serotonin or noradrenalin levels, by means of pharmacological pretreatment with PCPA (tryptophan hydroxylase inhibitor; Koe and Weissman, 1966) or DSP4 (noradrenergic neurotoxin; Grzanna et al., 1989), could attenuate or even block the behavioral effect of P2R antagonist in the FST. Altogether, these data could help understanding if monoamines would be involved in the mechanisms underlining the antidepressant-like effect induced by P2R blockade.

2. Results

2.1. Dose-response curves for FLX, RBX and PPADS treatment on mice submitted to FST or OFT

One-way ANOVA indicates a significant effect of drug treatment on immobility time in the FST [$F_{9,60} = 3.453$. $p < 0.05$]. Treatment with PPADS (6.25 mg/kg), FLX (20 mg/kg) and RBX (5 mg/kg) significantly decreased immobility time on FST (Dunn's *post hoc* test, $p < 0.05$), as seen in Fig. 1A. Effective doses of PPADS (6.25 mg/kg), FLX (20 mg/kg) and RBX (5 mg/kg) were not able to change locomotor behavior of animals tested on OFT (Fig. 1B – $F3_{,20} = 1.715$, non-significant – NS).

2.2. Add on effects of PPADS sub-effective dose to FLX or RBX sub-effective doses on FST and OFT

Two-way ANOVA indicates a significant treatment effect of first injection with FLX or RBX [$F_{2,38} = 7.831$; $p < 0.01$], of second injection with PPADS [$F_{1,38} = 4.534$; $p < 0.05$] and an interaction between factors [$F_{2,38} = 4.727$; $p < 0.05$]. In column comparison, combination of sub-effective doses of PPADS (3 mg/kg) and FLX (10 mg/kg) decreased immobility time compared to VEH/VEH ($p < 0.05$) and VEH/PPADS ($p < 0.01$), whereas combination of sub-effective doses of PPADS and RBX (2.5 mg/kg) decreased immobility time compared to VEH/VEH ($p < 0.01$), VEH/PPADS ($p < 0.001$) and RBX/VEH ($p < 0.05$) groups (Fig. 2A – $F5_{,38} = 5.915$). Combination of sub-effective doses of PPADS (3 mg/kg) and FLX (10 mg/kg) or RBX (2.5 mg/kg) did not change locomotor activity of animals on OFT (Fig. 2B – $F2_{,14} = 0.6730$, NS).

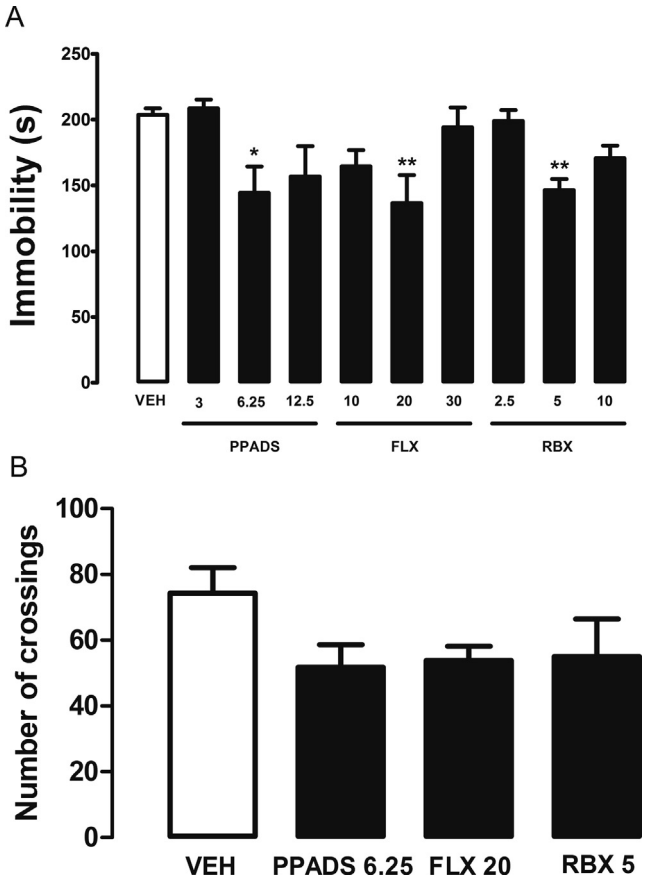

Fig. 1. Dose-response curves for FLX, RBX and PPADS treatment on mice FST and OFT. (A) PPADS (3, 6.25, 12.5), FLX (10, 20, 30), RBX (2.5, 5, 10) or vehicle (10 mL/kg) were administered 30 min before FST (n = 6, 7, 7, 7, 7, 5, 5, 7, 10, 9, respectively). Data are expressed as mean ± SEM of immobility time (s); $^{*}p < 0.05$, $^{**}p < 0.01$ from control group. (B) PPADS (6.25 mg/kg, n = 6), FLX (20 mg/kg, n = 6), RBX (5 mg/kg, n = 6) or VEH (n = 6) was administered 30 min before being submitted to OFT. Data are expressed as Mean ± SEM of total quadrant traveled in the OFT.