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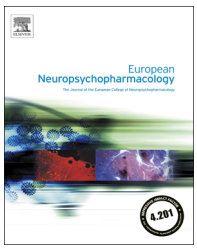

Antidepressant- and anticompulsive-like effects of purinergic receptor blockade: Involvement of nitric oxide

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Abstract
Activation of purinergic receptors by ATP (P2R) modulates glutamate release and the activation of post-synaptic P2R is speculated to induce nitric oxide (NO) synthesis. Increased glutamatergic and nitrergic signaling have been involved in the neurobiology of stress-related psychiatric disorders such as anxiety and depression. Therefore, the aim of this study was to test the effects of two P2R antagonists (PPADS and iso-PPADS) in animals submitted to models predictive of antidepressant-, anxiolytic- and anticompulsive-like effects. Swiss mice receiving PPADS at 12.5 mg/kg showed reduced immobility time in the forced swimming test (FST) similarly to the prototype antidepressant imipramine (30 mg/kg). This dose was also able to decrease the number of buried marbles in the marble-burying test (MBT), an anticompulsive-like effect. However, no effect was observed in animals submitted to the elevated plus maze (EPM) and to the open field test. The systemic administration of iso-PPADS, a preferential P2XR antagonist, also reduced the immobility time in FST, which was associated to a decrease in NO_x levels in the prefrontal cortex. In addition, P2X7 receptor was found co-immunoprecipitated with neuronal nitric oxide synthase (NOS1) in the prefrontal cortex. These results suggest that P2X7, possibly coupled to NOS1, could modulate behavioral responses associated to stress-related disorders and it could be a new target for the development of more effective treatments for affective disorders.

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1. Introduction

Psychiatric disorders have serious impact over individual life quality and, given their high incidence, comprise important socioeconomic consequences (World Health Organization, 2008). Several of these disorders, such as major depression and generalized anxiety, have been extensively studied, but their pathophysiology is still incompletely understood (Graeff and Zangrossi, 2010; Harmer et al., 2009). This is also true for obsessive-compulsive disorder (OCD), an anxiety-spectrum disorder with a poorly understood neurobiology (Andersen et al., 2010). A common feature among these disorders, however, is that antidepressant drugs based on monoaminergic mechanisms have been in the front line of the treatment options (Graeff and Zangrossi, 2010; Hindmarch, 2002). These drugs, however, have serious limitations, such as being ineffective in approximately 40% of the patients and the need of several weeks of treatment before a therapeutic effect can be observed (Hindmarch, 2002). Therefore, new pharmacological, non-monoaminergic based, targets have been studied with the aim of developing more effective treatment options.

The interest in adenosine 5′-triphosphate (ATP) as a signaling molecule in the central nervous system (CNS) has increased in the last years due to studies showing its involvement in the modulation of several physiological and pathological processes in the brain (Burnstock, 2006, 2007; Burnstock et al., 2011a, b). For instance, in the CNS, ATP signaling is crucial for postnatal development, synaptic plasticity, cognitive and behavioral functions, neuropathic pain, neuroinflammation, neuroprotection and neurodegeneration (Burnstock et al., 2011a; Khakh and North, 2006). This signaling is mediated by the activation of P2 receptors (P2R), which can be distinguished in two major families: a P2X family of ligand-gated ion channels and a P2Y family of G-protein-coupled receptors (Abbracchio and Burnstock, 1994). There are eight subtypes of P2Y receptors (P2YR 1, 2, 4, 6, 11, 12, 13, 14), which are widely expressed in epithelial, immune and glial cells but they can also be found in neurons (Burnstock, 2008). P2YR activation increases intracellular inositol trisphosphate (IP3), thus triggering the release of calcium ions from intracellular stores into the cytosolic compartment (Butt, 2011). P2X receptors (P2XR) are ligand-gated nonspecific cation channels, which increase intracellular calcium by allowing its direct influx (James and Butt, 2002). P2XR are subdivided into seven subtypes (P2X1-7) and are highly expressed in the CNS (Burnstock, 2008). Therefore, in general, the activation of P2R by ATP triggers the increase of intracellular calcium concentrations, which is a determinant factor in modulating neurotransmitter release.

Activation of P2XR facilitates neurotransmitter release, particularly of glutamate, and stimulates single-channels opening (Florenzano et al., 2008; North, 2002). The increase in glutamate release in response to P2XR activation has been described for different brain structures and it involves a presynaptic mechanism (Cho et al., 2010; Inoue et al., 1992; Khakh, 2009; Khakh and Henderson, 1998). In addition, the activation of the P2XR may also modulate the release of pro-inflammatory cytokines, such as IL-1beta, thus facilitating/aggravating neuroinflammatory conditions (Bernardino et al., 2008; Miller et al., 2011; Wilson et al., 2007). P2R are widespread in the mammalian nervous system, including the brain, spinal cord, sensory systems and autonomic ganglia. In the CNS they are found in regions involved in behavioral and emotional control, such as hippocampus, cerebral cortex, locus coeruleus and striatum, both in glial and neuronal cell types (Butt, 2011; Norenberg and Illes, 2000).

Increased glutamatergic neurotransmission have been implicated in several stress-related disorders, such as anxiety (Cortese and Phan, 2005; Simon and Gorman, 2006) and depression (Shors et al., 1989; Skolnick et al., 2009; Zarate et al., 2006). The increasing interest in this neurotransmission has been attributed to the fact that anti-glutamatergic drugs, such as riluzole, can be effective in depressive patients resistant to monoaminergic drugs (Zarate et al., 2010). Moreover, the glutamatergic NMDA receptor antagonist ketamine induces rapid and long-lasting antidepressant effects in clinical and preclinical models (Berman et al., 2000; Zarate et al., 2006). Significant anxiolytic-like effects have also been observed in response to ketamine and other anti-glutamatergic agents in animals and humans (Li et al., 2010; Machado-Vieira et al., 2012; Sanacora et al., 2012).

In addition to regulating glutamatergic neurotransmission, P2R might also modulate nitric oxide (NO) synthesis in the brain (Florenzano et al., 2008). Since the activation of NMDA receptors by glutamate leads to NO synthase (NOS) activation and NO synthesis (Garthwaite et al., 1989), Florenzano et al. (2008) proposed that purinergic mechanisms control nitric oxide (NO) levels by influencing NMDA receptors. However, there is also evidence that P2R and NOS1 are co-localized in hypothalamus, hippocampus and amygdaloid complex, therefore suggesting a direct involvement of the purinergic system in controlling NO synthesis in the brain (Kittner et al., 2003).

NO is thought to play an important role in the neurobiology of stress-related disorders (Jefferys and Funder, 1996; Joca and Guimaraes, 2006; Zhou et al., 2007). NOS inhibitors induce antidepressant- and anxiolytic-like effects in animal models such as the forced swimming (Beijamini et al., 1998; Jefferys and Funder, 1996; Joca and Guimaraes, 2006; Wegener et al., 2010) and the elevated plus maze tests (Forestiero et al., 2006; Spiacci et al., 2008).

Therefore, it is plausible to suggest that the pharmacological blockade of P2R receptors could elicit antidepressant- and/or anxiolytic-like effects. In fact, Basso et al. (2009) reported that P2X7 receptor knockout (KO) mice presented an antidepressant-like phenotype, but no behavioral change in anxiety tests. On the other hand, another study described contradictory results (Boucher et al., 2011). Since results obtained with knockout animals may be confounded by the absence of the investigated receptor along brain development, it would be important to examine the effects induced by the pharmacological blockage of P2R. However, no such study has been performed so far. In addition, a possible involvement of NO in these effects has not been investigated yet.

Based on the aforementioned evidence, the objective of this study was to investigate the effects induced by the pharmacological blockade of P2R in animals submitted to models predictive of antidepressant- and anxiolytic-like effects. Considering that NOS inhibitors, as well as antidepressants that block serotonin reuptake, present anti-compulsive properties (Krass et al., 2010; Umathe et al., 2009), we also studied if PR2 blockade would induce