# The receptor P2X7 participates in the AMPH-induced increase of proinflammatory environment and ultimately in the behavioral response in an animal model of mania

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Evidence points to increased levels of the pro-inflammatory cytokines TNF-alfa and IL-1beta in plasma and in postmortem frontal cortex from Bipolar Disorder (BD) patients compared with controls. The purinergic system, primarily the receptor P2X7 (P2X7R), has been implicated in the pathophysiology of medical conditions of central nervous system. This particular receptor, an adenosine 5'-triphosphate (ATP)-binding ligand-gated ion channel, is activated only by high concentrations of extracellular ATP and plays a key role in the modulation of the inflammatory response. In the present work, we aimed to explore the behavioral and inflammatory response in a pharmacological animal model of mania and the participation of the P2X7R in these processes. Male wild-type C57BL/6 (P2X7R+/+) and P2X7R knockout (P2X7R−/−) mice were used throughout this study. Mice received intraperitoneal (i.p.) injections of D-amphetamine (AMPH) (2 mg/kg) or vehicle once a day for a period of 7 days. On the 7th day of treatment, when appropriate, animals received a single intracerebroventricular (i.c.v.) microinjection of vehicle; potent P2X7R agonist (BzATP), 10.5 nmol; non-selective P2X7R antagonist (BBG), 20 nmol; and selective P2X7R antagonist (A438079), 1.75 nmol. After treatments we performed the open field behavioral testing for 1h. After that, animals were euthanized and striatum (STR), prefrontal cortex (PFC) and hippocampus (HPC) were isolated for biochemical analysis. The traveled time was analyzed by the ANY-maze software. The concentration of TNF-alfa and IL1-beta was determined by flow cytometry using the BD™ Cytometric Bead Array (CBA) Mouse Enhanced Sensitivity Flex Set. Two or one-way ANOVA, when appropriate, with Tukey post hoc test was employed to evaluate differences between groups. Results: AMPH significantly increased locomotor activity when compared to control animals ($p = 0.02$), also when associated with BzATP (0.03). The A438079 significantly reduced the locomotor activity to control levels when compared with the vehicle/AMPH and BzATP/AMPH groups ($p = 0.03$ and $p = 0.04$, respectively). Two-way ANOVA revealed a significant interaction between the effects of genotype and AMPH administration in the locomotor activity ($F = 4.814$; $df = 1$; $p = 0.042$). As expected, post hoc analysis has indicated that AMPH increased locomotor activity in P2X7R+/+ animals in comparison to the P2X7R+/+/vehicle control group ($p = 0.001$). AMPH had no effects in P2X7R−/− animals when compared to P2X7R−/−/vehicle controls ($p = 0.62$). There was a significant increase in IL-1β levels in STR induced by AMPH ($p = 0.003$), which was reversed by the treatment with BBG ($p = 0.005$) and A438079 ($p = 0.005$). In the HPC, IL-1beta levels were increased only with the co-treatment with BzATP and AMPH ($p = 0.025$), which was reversed by the treatment with A438079 ($p = 0.023$). Similarly, there was a significant increase in the levels of TNF-alfa ($p = 0.018$) in HPC induced by the cotreatment with BzATP and AMPH, which was reversed by the treatment with A438079 ($p = 0.044$). Our results suggest that the P2X7R participates in the AMPH-induced increase of proinflammatory environment and ultimately in the behavioral changes, demonstrated by the reversal role of P2X7R blockage on AMPH response and by the behavioral lack of responsiveness to AMPH observed in P2X7R-/- mice. In conclusion, our findings suggest that P2X7R has the potential to become a new therapeutic target in BD.

Keywords: Bipolar Disorder, P2X7 receptor, neuroinflammation