European Neuropsychopharmacology (▮▮▮▮) ▮, ▮▮▮–▮▮▮

www.elsevier.com/locate/euroneuro

Looking for prosocial genes: ITRAQ analysis of proteins involved in MDMA-induced sociability in mice

Konstantin Kuteykin-Teplyakov*, Rafael Maldonado

Laboratory of Neuropharmacology, Universitat Pompeu Fabra, Barcelona, Spain

Received 30 May 2014; received in revised form 25 July 2014; accepted 10 August 2014

KEYWORDS
Social behavior;
Proteomics;
N-Methyl-3;
4-methylenedioxyamphetamine;
Oxytocin;
5-HT1A receptor

Abstract
Social behavior plays a fundamental role in life of many animal species, allowing the interaction between individuals and sharing of experiences, needs, and goals across them. In humans, some neuropsychiatric diseases, including anxiety, posttraumatic stress disorder and autism spectrum disorders, are often characterized by impaired sociability. Here we report that N-Methyl-3, 4-methylenedioxyamphetamine (MDMA, "Ecstasy") at low dose (3 mg/kg) has differential effects on mouse social behavior. In some animals, MDMA promotes sociability without hyperlocomotion, whereas in other mice it elevates locomotor activity without affecting sociability. Both WAY-100635, a selective antagonist of 5-HT1A receptor, and L-368899, a selective oxytocin receptor antagonist, abolish prosocial effects of MDMA. Differential quantitative analysis of brain proteome by isobaric tag for relative and absolute quantification technology (iTRAQ) revealed 21 specific proteins that were highly correlated with sociability, and allowed to distinguish between entactogenic prosocial and hyperlocomotor effects of MDMA on proteome level. Our data suggest particular relevance of neurotransmission mediated by GABA B receptor, as well as proteins involved in energy maintenance for MDMA-induced sociability. Functional association network for differentially expressed proteins in cerebral cortex, hippocampus and amygdala were identified. These results provide new information for understanding the neurobiological substrate of sociability and may help to discover new therapeutic approaches to modulate social behavior in patients suffering from social fear and low sociability.

Abbreviations: 5-HT1A, serotonin receptor subtype 1a; 5-HT2C, serotonin receptor subtype 2c; FDR, false discovery rate; GHB, gamma-hydroxybutyrate; GPCR, G-protein coupled receptor; iTRAQ, isobaric tag for relative and absolute quantification; LC, liquid chromatography; MDMA, 3,4-methylenedioxymethamphetamine; MS, mass spectrometry; PSM, peptide-spectrum match; SERT, 5-HT transporter

*Correspondence to: Laboratory of Neuropharmacology, Department of Natural Sciences and Health, Universitat Pompeu Fabra, Carrer Dr. Aiguader 88, Barcelona, 08003, Spain. Tel.: +34 93 3160824/69 3038816; fax: +34 93 3160901.

E-mail address: konstantin.kuteykin@upf.edu (K. Kuteykin-Teplyakov).

http://dx.doi.org/10.1016/j.euroneuro.2014.08.007
0924-977X/

Please cite this article as: Kuteykin-Teplyakov, K., Maldonado, R., Looking for prosocial genes: ITRAQ analysis of proteins involved in MDMA-induced sociability in mice. European Neuropsychopharmacology (2014), http://dx.doi.org/10.1016/j.euroneuro.2014.08.007

1. Introduction

Social behavior plays a fundamental role in life of many animal species, allowing the interaction between individuals and sharing of experiences, needs, and goals across them (de Waal, 2011). Parental care, pair bonding, emotional convergence, cooperation and aggression are prototypical examples of these activities. Thus, sociability is not restricted to humans, but appears in many species (Decety et al., 2012). In humans, some neuropsychiatric diseases, such as anxiety, posttraumatic stress disorder, autism spectrum disorders, Asperger's syndrome and some cerebral lesions are often characterized by impaired sociability (Caldwell, 2012). The neurobiological substrate underlying sociability has not been yet fully clarified. Brain areas involved in social processes mainly include cortical and limbic structures that are important for cognitive and emotional functions (Blakemore, 2008). The amygdala and cortical areas, such as the posterior superior temporal sulcus, the temporal poles and the medial prefrontal cortex are considered to have a major role in social behavior (Fossati, 2012). Hippocampus is crucial in memory formation and also seems to be important in maintaining social behavior (Decety et al., 2012). Psychoactive drugs that promote social behavior are called "entactogens". Most entactogens are amphetamine derivates, phenethylamines or tryptamines that enhance serotonergic activity in the central nervous system, and the pharmacological properties of entactogens result in behavioral effects that are distinct from classical psychostimulants (Nichols et al., 1986). The first discovered and relatively well-studied entactogen is MDMA (N-Methyl-3,4-methylenedioxyamphetamine, also known as "Ecstasy"), a representative substance for revealing the pharmacological mechanisms of this class of drugs. In humans, MDMA produces euphoria, wellbeing, sociability, self-confidence, feelings of emotional closeness with others, reduced interpersonal defensiveness and extroversion (Vollenweider et al., 1998). Subjects with previously severe post-traumatic stress disorder, who were unresponsive to existing treatments, had symptomatic relief by MDMA-assisted psychotherapy (Mithoefer et al., 2013). However, MDMA and other similar entactogens cannot be used for long-term treatment due to the cardiovascular effects, hyperthermia and neurotoxicity in serotonergic terminals among other adverse effects. Therefore, there is strong demand to mimic prosocial effects of entactogens by other approaches that would be, suitable for treatment of patients suffering from social fear and low sociability. For this purpose, it would be crucial to determine the molecular substrates of these prosocial effects of MDMA. The mechanisms involved in the adverse effects of MDMA have been intensively studied over last decades (de la Torre et al., 2004). However, the mechanism of MDMA entactogen-induced sociability remains unclear. The monoamine neurotransmitter serotonin, and two neuropeptides, oxytocin and vasopressin have been consistently linked with the neural regulation of sociability in mammals (Caldwell, 2012). MDMA at a moderate dose (5 mg/kg) increases sociability, promotes the release of oxytocin and activates hypothalamic oxytocin-containing neurons in rats via an action on serotonin-1A (5-HT1A) receptors (Hunt et al., 2011; Thompson et al., 2007). The influence of MDMA on oxytocin release and its correlation with subjective prosocial feelings has also been demonstrated in humans (Dumont et al., 2009), as well as the impact of 5-HT1A receptor activation in subjective MDMA effects (Hasler et al., 2009). Few studies have already reported the effects of high doses and/or chronic administration of MDMA on brain transcriptome and proteome in rodents (Thiriet et al., 2002; Martinez-Turrillas et al.,2006; Benturquia et al., 2008; van Nieuwenhuijzen et al., 2010; Fernandez-Castillo et al., 2012). However, the data concerning changes of gene/protein expression in the brain after single administration of a low dose of MDMA, relevant to MDMA doses producing entactogenic effects in humans are absent. Most of the previous studies investigating MDMA side effects have evaluated the alterations in transcriptome and proteome many hours (or even days/weeks) after MDMA administration, when entactogenic effect already disappeared. Since the goal of the present study was to investigate the substrate of MDMA of prosocial effect, the proteomic analysis was carried out one hour after MDMA administration, when the behavioral effect of the drug reach the maximum level. Indeed MDMA plasma concentration and behavioral effects reach their maximum in 10 min after systemic administration (*i.p.*) in mice injection and both significantly decrease 90 min later (Fantegrossi et al., 2009). Considering the large availability of genetically modified mice and the difficulties to carry out similar research in humans, the mouse seems the most convenient species to evaluate the genes and/or proteins involved in sociability.In this study, we used a novel behavioral mouse model of sociability by co-administration of relatively low dose of MDMA together with WAY-1000635, an antagonist for the 5-HT1A receptor, or with L-368899, an oxytocin receptor antagonist, that allowed to distinguish between entactogenic and adverse effects of MDMA. Considering the significant mismatch between the levels of mRNAs and the corresponding proteins (see review of Vogel, Marcotte, 2012), we decided to omit transcriptomic approaches focusing our research on the proteins correlated with behavioral alterations. Therefore, quantitative proteomic analysis of variations in protein expression in cerebral cortex, hippocampus and amygdala from mice exposed to these treatments were used to shed light onto the molecular mechanisms underlying social behavior, which may be helpful to identify new targets for the treatment of social disorders.

2. Experimental procedures

2.1. Animals

C57BL/6J mice were purchased from Charles River and were handled for 1 week before starting the experiments. Male mice aged 8–12 weeks were housed four per cage in a temperature (21±1 °C) and humidity-controlled (55±10%) environment. Food and water were available *ad libitum*. All the experiments were performed during the dark phase of a 12 h light, 12 h dark cycle (reverse lighting cycle: light on at 8 p.m. and off at 8 a.m.) under the sodium lamp (McLennan and Taylor-Jeffs, 2004). All animal procedures followed standard ethical guidelines (European Union Directive 2010/63/EU) and were approved by the local ethical committee (Comitè Ètic d'Experimentació Animal – Parc de Recerca Biomèdica de Barcelona, CEEA-PRBB).

2.2. Drugs and treatments

All drugs and laboratory reagents were obtained from Sigma-Aldrich, if no otherwise specified. MDMA (±3,4-methylenedioxy-N-methylamphetamine; 1-(1,3-benzodioxol-5-yl)-N-methylpropan-2-amine or

Please cite this article as: Kuteykin-Teplyakov, K., Maldonado, R., Looking for prosocial genes: ITRAQ analysis of proteins involved in MDMA-induced sociability in mice. European Neuropsychopharmacology (2014), http://dx.doi.org/10.1016/j.euroneuro.2014.08.007