Neuropharmacology 133 (2018) 366–374

Contents lists available at ScienceDirect

Neuropharmacology

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

Melanocortin 4 receptor stimulation improves social deficits in mice through oxytocin pathway

Andrea Mastinu [a, *], Marika Premoli [a], Giuseppina Maccarinelli [a], Mariagrazia Grilli [b], Maurizio Memo [a], Sara Anna Bonini [a]

[a] Department of Molecular and Translational Medicine, University of Brescia, 25123 Brescia, Italy
[b] Laboratory of Neuroplasticity, Department of Pharmaceutical Sciences, University of Piemonte Orientale, 28100 Novara, Italy

ARTICLE INFO

Article history:
Received 20 June 2017
Received in revised form
6 February 2018
Accepted 9 February 2018
Available online 15 February 2018

Keywords:
THIQ
Melanocortin 4 receptor
Oxytocin
p50 KO mouse
Social behaviour

ABSTRACT

Several studies on humans and mice support oxytocin's role in improving social behaviour, but its use in pharmacotherapy presents some important limiting factors. To date, it is emerging a pharmacological potential for melanocortin 4 receptor (MC4R) agonism in social deficits treatment. Recently, we demonstrated that the deletion of the *NFKB1* gene, which encodes the p50 NF-κB subunit, causes impairment in social behaviours, with reductions in social interactions in mice. In this work, we tested the acute effects of THIQ, a selective melanocortin 4 receptor (MC4R) agonist. THIQ treatment increased social interactions both in wild type and $p50^{-/-}$ mice. In particular, after treatment with THIQ, $p50^{-/-}$ mice showed a prosocial behaviour analogous to that of basal WT mice. Moreover, intranasal treatment with an oxytocin antagonist blocked social interactions induced by THIQ, demonstrating that its prosocial effects are mediated by the oxytocin pathway. The data obtained reinforce using MC4R agonists to ameliorate social impairment in NDDs.

1. Introduction

Social interaction is a primary and adaptive behavioural component of mammalian species. For social animals, the interaction is important for the organization and stability of societies to define social hierarchy and for mate choice (Berry and Bronson, 1992). Several neurodevelopmental disorders (NDDs) such as schizophrenia and autism are characterized by social behavioural deficits. NDDs include developmental brain dysfunction characterized by neuropsychiatric deficits generating impairments in motor function, learning, verbal or non-verbal communication, social, or occupational functioning. All these phenotypic alterations have neuroanatomical basis. Indeed, increased cortical layers thickness, an abnormal columnar organization, cortex misconnections are often associated with NDDs (Innocenti et al., 2003; Budday et al., 2015). Recently, we observed specific neurodevelopmental alterations in a genetic mouse model, namely the NF-κB p50 knock out (p50 KO) mouse (Bonini et al., 2016). p50 KO mice were generated by targeted deletion of the *NFKB1* gene that encodes for the precursor of the p50 NF-κB subunit. NF-κB is involved in many physiological functions such as immune and inflammatory responses, cell survival and death (Grilli and Memo, 1999; Kucharczak et al., 2003), in cell plasticity and morphology remodelling (Mattson, 2005; Gutierrez and Davies, 2011; Bonini et al., 2011). This mouse model has been widely used in studies on inflammation, immunity, metabolic diseases and cancer (de Valle et al., 2016; Minegishi et al., 2015; Southern et al., 2012). Moreover, we found that adult p50 KO mice display abnormal columnar organization in the somatosensory cortex and altered neurite orientation, associated with hyperactivity and impairment in social behaviours with a reduction in social interactions (Bonini et al., 2016). All these cortical and behavioural alterations have also been reported in different NDD models, including models of autism spectrum disorders (ASD) and schizophrenia (Casanova et al., 2006, Casanova and Tillquist, 2008; Beasley et al., 2009; Stoner et al., 2014; Gaudissard et al., 2017).

Drugs currently used for NDDs are mainly symptomatic and often are ineffective for social deficits (Ji and Findling, 2016). Therapeutic intervention commonly aims to reduce psychotic symptoms and relies on atypical antipsychotics, especially risperidone, aripiprazole and olanzapine (Lamberti et al., 2016; Mastinu

* Corresponding author. Department of Molecular and Translational Medicine, University of Brescia, Viale Europa, 11, 25123 Brescia, Italy.
E-mail address: andrea.mastinu@unibs.it (A. Mastinu).

https://doi.org/10.1016/j.neuropharm.2018.02.007
0028-3908/

et al., 2012; Lazzari et al., 2017). No current medical treatments exist for prevention of NDDs and for amelioration of social deficits. Indeed, risperidone treatment in p50 KO mice resulted in decreased hyperactivity but had no effect on social deficits (Bonini et al., 2016). A promising compound to rescue social impairments in NDDs is represented by oxytocin (Woolley et al., 2014; Gordon et al., 2013; Kirsch, 2015). Oxytocin is synthesized in the neurons of the paraventricular nuclei (PVN) and supraoptic nuclei (SON) of the hypothalamus and released in the brain or in the blood (Ludwig and Leng, 2006). Oxytocin exerts several neuroendocrine functions when released in the peripheral bloodstream and modulates social behaviour when released in the brain. In particular, oxytocin has been reported to increase social stimuli, to promote parental nurturing and social bonds in mouse models of ASD (Peñagarikano et al., 2015; Teng et al., 2016). Furthermore, intranasal oxytocin improved social deficits both in patients with schizophrenia/schizoaffective disorder (Shilling and Feifel, 2016) and in ASD patients (Andari et al., 2010; Guastella et al., 2010). Studies in humans support the pharmacological role of oxytocin in stress, anxiety disorders, social phobia, postpartum depression, and bipolar disorder, with a positive action on reward in social behaviour and communication (MacDonald et al., 2013; Singer et al., 2008). Lastly, oxytocin is involved also in peripheral inflammatory responses mediated by NF-κB, as previously reported (Soloff et al., 2006; Kim et al., 2015). In particular, Soloff and colleagues hypothesize that the NF-κB p65 subunit interacts with the promoter of the oxytocin receptor and down regulates its expression. At present the specific mechanisms of p65 interaction with the oxytocin receptor promoter remains considerably complex.

Despite these encouraging results both in animal models and in humans, the use of oxytocin in pharmacotherapy presents some relevant limitations. First of all, oxytocin is a peptide therefore it cannot be used by oral administration. Another adverse factor is represented by oxytocin's short half-life. Indeed, oxytocin degrades very rapidly by intravenous administration, resulting in short-lasting effects (Modi et al., 2016). Furthermore, its brain penetrance is uncertain; indeed, some studies report that only a small fraction can pass the blood brain barrier and enter the brain (Mens et al., 1983; Guastella et al., 2013). On the contrary, Lee and colleagues demonstrated cerebrospinal fluid penetrance of exogenous oxytocin administered peripherally, by intranasal or intravenous route (Lee et al., 2018). Intranasal delivery resulted to be the more efficient pathway for oxytocin compared to intravenous administration, but unfortunately it presented wide variability of both cerebrospinal fluid and plasma concentrations between different treated animals (Lee et al., 2018). Hence, it is not a manageable treatment.

To circumvent these problems, compounds able to enhance endogenous oxytocin release can be used. Sabatier et al. (2003) showed that α melanocyte-stimulating hormone (αMSH) has a selective modulatory action on SON oxytocin neurons via melanocortin 4 receptor (MC4R) agonism. Moreover, MC4R agonist treatment increases oxytocin release in the brain (Sabatier et al., 2003) and promotes partner preference in voles (Modi et al., 2015). THIQ is a selective and potent MC4R agonist; it is an orally active small non-peptide MC4R mimetic compound (Grunewald et al., 1999). In addition, THIQ can penetrate the blood-brain barrier (BBB), as previously reported by an in vitro BBB permeability study (Grunewald et al., 1999). Moreover, it shows chemical properties of log P 5.65 ± 0.93, calculated using Advanced Chemistry Development (ACD/Labs) Software V11.02. THIQ has already been reported to induce MC4R-mediated erectogenic effects in both mice and rats with sexual dysfunctions (Martin et al., 2002).

To identify a correlation between social deficit and the oxytocin pathway in p50 KO mice, we initially measured the oxytocinergic tone. Then, we evaluated the THIQ effect on social deficits and whether this effect was mediated by oxytocin pathway.

2. Materials and methods

2.1. Animals

Experiments were conducted in conformity with the European Communities Council Directive of 1986 (86/609/EEC), approved by the Italian Ministry of Health, and the Animal Care and Use Committee of the University of Brescia. Animals were housed two-to-three per cage in a 12 h light/dark cycle (light phase from 8:00 a.m. to 8:00 p.m.) with food and water available ad libitum. The cage size was 15 cm wide x 35 cm long x 12 cm deep. Temperature (22 °C) and humidity (50% ± 10) in the cages were automatically regulated by the Sealsafe Aero System by individually ventilated cages with EPA filters (Tecniplast Group, Italy). NF-κB $p50^{-/-}$ mice (B6; 129P2-Nfkb 1tm 1 Bal/J) and wild-type mice (B6; 129PF2) were purchased from The Jackson Laboratories (Bar Harbor, ME, USA). Stable mating couples were maintained to amplify the colony and the progeny was used for the experiments. In particular, wild-type (WT) and $p50^{-/-}$ (p50 KO) age-matched mice (4-6-month-old mice; weight = 25–35 g) were used. All the experiments were performed on male mice.

2.2. Drugs and pharmacological treatment

For pharmacological treatment, melanocortin 4 receptor agonist THIQ (*N*-[(1*R*)-1-[(4-Chlorophenyl)methyl]-2-[4-cyclohexyl-4-(1*H*-1,2,4-trazol-1-ylmethyl)-1-piperidinyl]-2-oxoethyl]-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide, Tocris Bioscience, Bristol, UK) was administered acutely via intraperitoneal injection 30 min before the behavioural tests, according to previously reported data (Peñagarikano et al., 2015). THIQ doses were 0.5, 1, and 2 mg/kg in a 10 mL/kg volume, 250 μL for an average 25 g mouse and were chosen on the basis of previous data reporting K_i, IC_{50} and MC4R activation (Martin et al., 2002; Sebhat et al., 2002). Oxytocin receptor antagonist, L-371,257 (Tocris Bioscience, Bristol, UK) was intranasally administered at a dose of 300 μg/kg; a drop of a 2.5 μL solution was placed in the animal nostril with a P10 pipette to induce the mouse to aspirate the drop into its nasal cavity. The mice received L-371,257 15 min before THIQ administration (Peñagarikano et al., 2015). All drugs, suspended in 0.9% saline solution, were prepared daily, sonicated, and administered.

2.3. Radioimmunoassay

To correlate oxytocin levels with THIQ treatment, we measured hypothalamic oxytocin levels 30 min after intraperitoneal drug administration as reported previously (Peñagarikano et al., 2015). Mice (6 for WT and 5 for the p50 KO mice group) were sacrificed and hypothalamus was isolated, weighted and homogenized with acetic acid (Sigma Aldrich, Milano, Italy) 50% v/v. Then, the tissue homogenate was boiled for 10 min at 100 °C and centrifuged at 12000 rpm for 30 min at 4 °C. One mL of supernatant was combined with 1 mL of Buffer A (Phoenix Pharmaceuticals, Burlingame, CA, USA) and centrifuged at 12000×*g* for 20 min, and we collected the supernatant. After, the samples were extracted onto the C-18 sep column (Phoenix Pharmaceuticals, Burlingame, CA, USA). The eluted sample was frozen at least 3 h before being placed in the lyophilizer. The lyophilized sample was reconstituted with one x assay buffer for RIA detection (Phoenix Pharmaceuticals, Burlingame, CA, USA). Briefly, the standard peptide, the antibody, the positive control, and unknown samples were reconstituted, and RIA buffer was diluted with 150 mL of distilled water. Dilutions of the