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Reduced oxytocin receptor gene expression and binding sites in different brain regions in schizophrenia: A post-mortem study

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ABSTRACT

Schizophrenia is a severe neuropsychiatric disorder with impairments in social cognition. Several brain regions have been implicated in social cognition, including the nucleus caudatus, prefrontal and temporal cortex, and cerebellum. Oxytocin is a critical modulator of social cognition and the formation and maintenance of social relationships and was shown to improve symptoms and social cognition in schizophrenia patients. However, it is unknown whether the oxytocin receptor is altered in the brain.

Therefore, we used qRT-PCR and Ornithine Vasotocin Analog ([^{125}I]OVTA)-based receptor autoradiography to investigate oxytocin receptor expression at both the mRNA and protein level in the left prefrontal and middle temporal cortex, left nucleus caudatus, and right posterior superior vermis in 10 schizophrenia patients and 6 healthy controls. Furthermore, to investigate confounding effects of long-term antipsychotic medication we treated rats with clozapine or haloperidol for 12 weeks and assessed expression of the oxytocin receptor in cortical and subcortical brain regions.

In schizophrenia patients, we found a downregulation of oxytocin receptor mRNA in the temporal cortex and a decrease in receptor binding in the vermis. In the other regions, the results showed trends in the same direction, without reaching statistical significance. We found no differences between antipsychotic-treated rats and controls.

Downregulated expression and binding of the oxytocin receptor in brain regions involved in social cognition may lead to a dysfunction of oxytocin signaling. Our results support a dysfunction of the oxytocin receptor in schizophrenia, which may contribute to deficits of social cognition.

1. Introduction

Schizophrenia is a severe psychiatric disorder that shows a chronic course and residual symptoms in >50% of patients (Marengo, 1994; Lambert et al., 2010). Despite treatment with antipsychotics, persisting negative and cognitive symptoms are frequent and lead to an unfavorable social prognosis (Green, 1996). Besides deficits in episodic and working memory, patients show impairments in social cognition, i.e. in psychological processes involved in the perception, encoding, storage, retrieval, and regulation of information about people in the social environment. Patients often display deficits in identifying emotions, inferring people's thoughts and managing their emotional reaction to others. These disturbed processes may lead to misinterpretation of the social intentions of others, social withdrawal, and impaired social functioning (Green et al., 2015).

Recently, interest has grown in oxytocin (OXT), a critical modulator of social cognition and the formation and maintenance of social relationships (Hurlemann and Scheele, 2016). OXT, a 9-amino acid neuropeptide, is known to be synthesized in the hypothalamus and released into the blood stream by axon terminals in the posterior pituitary. It is hypothesized to be released also from dendrites of hypothalamic

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neurons and to subsequently reach other brain regions by diffusion (Ludwig and Leng, 2006). In healthy volunteers, OXT has been shown repeatedly to reduce anxiety (Neumann and Slattery, 2016) and improve different facets of social cognition such as emotion recognition (e.g. of facial expression), Theory of Mind, social perception, empathic ability, and trust (Meyer-Lindenberg et al., 2011; Bartholomeusz et al., 2015). Considering these effects, a dysfunction of OXT and its 7-transmembrane G-protein-coupled receptor (OXTR) has been hypothesized in psychiatric disorders with impaired social cognition, such as schizophrenia and autism (Zik and Roberts, 2015; Guastella and Hickie, 2016). Augmentation strategies with intranasal OXT, which acts as an antipsychotic-like substance, have been developed in animal models relevant for schizophrenia (Lee et al., 2005; Peters et al., 2014), such as OXT- and OXTR-deficient mice (Caldwell et al., 2009; Chini et al., 2014), and are currently under investigation in schizophrenia patients. Such strategies have been shown to improve general and negative symptoms (Oya et al., 2015), social cognition (Pedersen et al., 2011), and emotion recognition (Averbeck et al., 2012). In addition, verbal memory was improved in schizophrenia patients after 3 weeks of intranasal OXT in a double-blind, placebo-controlled crossover study (Feifel et al., 2012), pointing to additional effects on other cognitive domains. Because of the short half-life and poor blood-brain barrier penetration of OXT, selective agonists of the OXTR have been developed, such as WAY-267464. These selective agonists have been shown to reverse deficits in prepulse inhibition of the acoustic startle reflex induced by MK-801 or amphetamine (Ring et al., 2010). Despite these findings in animal models, to date no clinical trials of OXTR agonists have been performed in humans.

Polymorphisms in the *OXT* and *OXTR* genes have been associated with schizophrenia (Souza et al., 2010a; Teltsh et al., 2012; Montag et al., 2013). Results are contradictory (Haram et al., 2015), but an association has been reported with negative symptoms and social withdrawal, overall symptom severity, and response to clozapine treatment (Souza et al., 2010b; Haram et al., 2015) as well as with a social cognition index (Davis et al., 2014).

In schizophrenia patients, higher OXT plasma levels have been reported to be associated with less severe positive symptoms and better pro-social functions (Rubin et al., 2010). Furthermore, OXT has been shown to interact with several neurotransmitters, such as dopamine, gamma-butyric acid (GABA), and serotonin (Rosenfeld et al., 2011; Rich and Caldwell, 2015; Mier and Kirsch, 2016). While in rodents OXTR is abundantly expressed in several brain regions (Elands et al., 1988), in the human brain its expression is more restricted, with the highest expression being found in the basal nucleus of Meynert, diagonal band of Broca and lateral septal nucleus (Loup et al., 1991), as assessed by receptor autoradiography. Furthermore, OXTR immunoreactivity in humans was found in regions such as the hypothalamus, anterior cingulate cortex, olfactory nucleus, and amygdala (Boccia et al., 2013). Recently, cortical brain regions such as the prefrontal and superior temporal cortex and the cerebellum were linked to social cognitive dysfunction in schizophrenia (Bartholomeusz et al., 2015; Bicks et al., 2015; Mothersill et al., 2015). Activation in these regions and the prefrontal cortex was shown to correlate with plasma OXT levels during a social perception task (Lancaster et al., 2015). Activation of the amygdala during processing of emotionally salient cues was affected by a variant in the *OXTR* gene in healthy volunteers (Tost et al., 2010). A meta-analysis of functional magnetic resonance imaging (fMRI) studies revealed hyperactivation of bilateral temporal lobes and left insula after intranasal OXT administration (Wigton et al., 2015). The nucleus caudatus is known to influence social behavior by driving the evaluation and interpretation of choices and their associated values (Adolphs, 2003). In a focal nucleus caudatus lesion, loss of empathy and difficulties in recognizing emotions in others, which is part of Theory of Mind, have been detected (Kemp et al., 2013). In schizophrenia patients, a lower activation of this region has been shown during trust games (Gromann et al., 2013). In a meta-analysis of structural magnetic resonance imaging (sMRI) studies, the volumes of the prefrontal and temporal cortex have been reported to be reduced in schizophrenia patients (Shepherd et al., 2012; Haijma et al., 2013). Additionally, a disturbed prefronto-thalamo-cerebellar circuit has been proposed to play a role in the pathophysiology of schizophrenia (Andreasen et al., 1999), and a decreased volume of the posterior superior cerebellar vermis has been reported (Okugawa et al., 2007; Segarra et al., 2008; Cohen et al., 2012).

Thomas Insel (2016), from 2002 until November 2015 the head of the National Institute of Mental Health, recently stated that "Without a clear picture of the landscape of oxytocin receptors in the human brain, clinical studies cannot be linked rigorously to preclinical research." In this post-mortem study in schizophrenia patients and healthy controls, we aimed to increase knowledge in this area by investigating OXTR expression at both mRNA expression and receptor binding sites. We intended to examine brain regions that are known to be involved in social cognition and behavior and the pathophysiology of schizophrenia, such as the prefrontal and temporal cortex, cerebellar vermis, and nucleus caudatus (Falkai et al., 2011; Bartholomeusz et al., 2015).

2. Material and methods

2.1. Human post-mortem tissue samples

Post-mortem brain samples from 10 inpatients with DSM-IV residual schizophrenia (6 males, 4 females; mean (standard deviation, SD) age 68.2 (5.0) years; mean (SD) post-mortem interval (PMI) 20.5 (3.6) hours; mean (SD) duration of disease 41.4 (4.1) years; mean (SD) age at onset 26.0 (2.4) years) were obtained at the Department of Neuropathology, Psychiatric Center Nordbaden, Wiesloch, Germany. Post-mortem brain samples from 6 healthy controls (5 males, 1 females; mean (SD) age 61.8 (6.8) years; PMI 15.7 (2.3) hours) were obtained during autopsies performed at the Institute of Neuropathology, University of Heidelberg, Germany (Table 1). Autopsy consent had been obtained from the donor or was given by a family member. Gray matter blocks of the left anterior prefrontal cortex (Brodmann area 10, BA10), left posterior medial temporal cortex (Brodmann area 21, BA21), nucleus caudatus (NC), and the right cerebellar posterior superior vermis was dissected by an experienced neuropathologist according to a brain atlas (Nieuwenhuys et al., 2008) (see also Fig. 1), snap frozen in liquid nitrogen-cooled isopentane, and stored at −80 °C until use. At least 3 blocks were obtained from each brain region. Sections and mRNA extraction were performed in the same sub-block of the brain regions with a size of 0.4–0.7 cm^3.

Experienced psychiatrists at the Wiesloch hospital collected a complete clinical history and diagnosis for all patients. Chlorpromazine equivalents (CPE) were used to assess cumulative doses of medication during the last ten years of the patients' lives (Rey et al., 1989; Woods, 2003). The last mean (SD) CPE dose was 283.3 (84.1) g, and the mean (SD) cumulative CPE dose in the last ten years was 3.4 (0.9) kg. Patients and healthy controls had not been treated with corticosteroids or estrogens prior to death. Neuropathological examinations were performed to exclude neurovascular or neurodegenerative disorders such as vascular dementia or Alzheimer's disease; Braak staging of neurodegeneration (Braak et al., 2006) was 2 or less in all patients and controls. Neither patients nor controls had a history of alcohol or drug abuse or severe physical illness (e.g. carcinoma), and none of the controls had a history of psychiatric disorders. All assessments and post-mortem evaluations and procedures were approved by the Ethics Committee of the Faculty of Medicine, University of Heidelberg, Germany and the Institutional Review Board (IRB, 009–238-MA).

2.2. Animal model: clozapine and haloperidol treatment

Three groups of 10 male Sprague Dawley rats (Taconic, Denmark) were fed ground pellets (Altromin maintenance diet with 19% crude protein, 4% crude fat, and 15% additional fat). From postnatal day (PD)

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