Psychoneuroendocrinology 62 (2015) 36–46

Contents lists available at ScienceDirect

Psychoneuroendocrinology

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

Activation of presynaptic oxytocin receptors enhances glutamate release in the ventral hippocampus of prenatally restraint stressed rats

Jérôme Mairesse[a,g], Eleonora Gatta[a,g], Marie-Line Reynaert[a,g], Jordan Marrocco[b,g], Sara Morley-Fletcher[a,g], Marion Soichot[c], Lucie Deruyter[a,g], Gilles Van Camp[a,g], Hammou Bouwalerh[a,g], Francesca Fagioli[d], Anna Pittaluga[e], Delphine Allorge[c], Ferdinando Nicoletti[a,f,g,1], Stefania Maccari[a,g,*,1]

[a] Univ.Lille, CNRS, UMR 8576, UGSF, Unité de Glycobiologie Structurale et Fonctionnelle, 59000 Lille, France
[b] Laboratory of Neuroendocrinology, The Rockefeller University, 10065 New York, NY, USA
[c] Faculté de Médecine de Lille, UDSL, 59045 Lille, France
[d] Azienda Sanitaria Locale, RM.E. UnitaÒperativa Complessa Adolescent, 00100 Rome, Italy
[e] Dept of Pharmacy, Univ. of Genoa, 16126 Genoa, Italy
[f] IRCCS Neuromed, 86077 Pozzilli, Italy
[g] LIA France/Italy (International Associated Laboratory "Prenatal Stress and Neurodegenerative Diseases", Glycobiology of Stress-related Diseases team, UMR 8576, UGSF, Unité de Glycobiologie Structurale et Fonctionnelle, 59000 Lille, France; Neuromed, 86077-Pozzilli, Italy and Sapienza University of Rome, 00185-Rome, Italy

ARTICLE INFO

Article history:
Received 22 January 2015
Received in revised form 3 July 2015
Accepted 7 July 2015

Keywords:
Perinatal stress
Carbetocin
Anxiety
Social behavior
Glutamate
Ventral hippocampus

ABSTRACT

Oxytocin receptors are known to modulate synaptic transmission and network activity in the hippocampus, but their precise function has been only partially elucidated. Here, we have found that activation of presynaptic oxytocin receptor with the potent agonist, carbetocin, enhanced depolarization-evoked glutamate release in the ventral hippocampus with no effect on GABA release. This evidence paved the way for examining the effect of carbetocin treatment in "prenatally restraint stressed" (PRS) rats, i.e., the offspring of dams exposed to repeated episodes of restraint stress during pregnancy. Adult PRS rats exhibit an anxious/depressive-like phenotype associated with an abnormal glucocorticoid feedback regulation of the hypothalamus-pituitary-adrenal (HPA) axis, and, remarkably, with a reduced depolarization-evoked glutamate release in the ventral hippocampus. Chronic systemic treatment with carbetocin (1 mg/kg, i.p., once a day for 2–3 weeks) in PRS rats corrected the defect in glutamate release, anxiety- and depressive-like behavior, and abnormalities in social behavior, in the HPA response to stress, and in the expression of stress-related genes in the hippocampus and amygdala. Of note, carbetocin treatment had no effect on these behavioral and neuroendocrine parameters in prenatally unstressed (control) rats, with the exception of a reduced expression of the oxytocin receptor gene in the amygdala. These findings disclose a novel function of oxytocin receptors in the hippocampus, and encourage the use of oxytocin receptor agonists in the treatment of stress-related psychiatric disorders in adult life.

1. Introduction

The function of oxytocin in the CNS has been the subject of extensive investigation since the early discovery that this hormone influences learning and memory processes (De Wied, 1965). Oxytocin has prominent effects on social behavior and anxiety (Ferguson et al., 2001; Jin et al., 2007; De Dreu et al., 2010; Insel, 2010; Labuschagne et al., 2010), and has potential clinical applications in psychiatric disorders, such as schizophrenia, post-traumatic stress disorder, addiction, and autism (Bartz and

* Corresponding author at: LIA-PSND Glycobiology of Stress-related Diseases Team, Department of Glycobiology, UMR8576 University of Lille1/CNRS, 59655-Villeneuve d'Ascq, France.
E-mail address: stefania.maccari@univ-lille1.fr (S. Maccari).
[1] Co-last authors.

http://dx.doi.org/10.1016/j.psyneuen.2015.07.005
0306-4530/

Hollander, 2006; Andari et al., 2010; Olff et al., 2010). The prevailing view is that oxytocin treatment in humans improves many aspects of social cognition and behavior, including interpersonal trust (Kosfeld et al., 2005; De Dreu et al., 2011), generosity (Zak et al., 2007), social recognition memory (Rimmele et al., 2009), and emotional empathy (Hurlemann et al., 2010). However, oxytocin may facilitate social interaction, even if detrimental, as in case of impeded trust and cooperation, or closeness in insecurely or anxiously attached individuals (Bartz et al., 2010, 2011). To explain these findings, it has been suggested that oxytocin mainly acts as an anti-stress hormone (Windle et al., 1997, 2004; Bartz et al., 2011). Oxytocinergic neurons are mainly found in the hypothalamus, whereas oxytocin receptors are widespread in the CNS, and are found in brain regions implicated in stress response and emotional processing such as the amygdala, ventral hippocampus, and nucleus accumbens (Stoop, 2012). In the hippocampus, oxytocin receptors are found predominantly in the soma and dendrites of GABAergic interneurons and their activation increases the firing of interneurons, thereby suppressing the activity of pyramidal neurons (Mühlethaler et al., 1984; Zaninetti and Raggenbass, 2000). In a recent manuscript, Owen et al. (2013) have demonstrated that activation of oxytocin receptors in the hippocampus enhances the activity of fast-spiking GABAergic interneurons, thus improving information processing. Oxytocin receptors are also found in hippocampal synaptosomes (Audigier and Barberis, 1985), but their function is still unknown.

Abnormalities in hippocampal synaptic transmission and plasticity lie at the core of the pathological phenotype induced by prenatal stress (Yaka et al., 2007; Mairesse et al., 2012; Marrocco et al., 2012, 2014). Prenatally restraint stressed (PRS) rats, i.e. the offspring of dams exposed to multiple episodes of restraint stress during pregnancy, represent an experimental animal model of anxious-depressive disorder endowed with face, construct, and pharmacological validity. These rats display anxiety- and depressive-like behaviors and show an excessive glucocorticoid response to acute stress, which is indicative of a dysregulation of the hypothalamus-pituitary-adrenal (HPA) axis caused by an impaired hippocampal glucocorticoid negative feedback (Maccari et al., 1995; Darnaudéry and Maccari, 2008). All these effects are reversed by chronic antidepressant medication (Mairesse et al., 2013; Marrocco et al., 2014). Interestingly, PRS rats show a selective reduction in glutamate release in the ventral hippocampus, which is causally related to the anxious-/depressive-like phenotype of PRS rats. Chronic treatment with fluoxetine or agomelatine, which produced antidepressant and anxiolytic effects in PRS rats, also corrected the defect in glutamate release in the ventral hippocampus in these rats (Marrocco et al., 2014). In addition, intrahippocampal injection of a cocktail of two drugs that enhance glutamate release (e.g. the type 2/3 metabotropic glutamate receptor antagonist, LY341495, and the $GABA_B$ receptor antagonist, CGP52432) abolished anxiety-like behavior in PRS rats (Marrocco et al., 2012).

Thus, the phenotype of PRS rats is particularly appropriate for the study of novel anti-stress drugs with mechanisms of action based on the modulation of hippocampal glutamatergic transmission.

Here, we examined whether activation of presynaptic oxytocin receptors could modulate glutamate release in the ventral hippocampus and whether, as a consequence of this mechanism, it could correct the pathological phenotype of PRS rats. To activate oxytocin receptors we used carbetocin, a potent and selective oxytocin receptor agonist, which has longer elimination half-life than oxytocin (Cort et al., 1981), and is clinically used for the control of postpartum bleeding (Engstrøm et al., 1998). Carbetocin can cross the blood-brain barrier (Dvorská et al., 1992), and is known to affect animal behavior after peripheral administration (Klenerova et al., 2009, 2010; Chaviaras et al., 2010; Mak et al., 2012).

2. Materials and methods

2.1. Animals

Nulliparous female Sprague-Dawley rats weighing 250–260 g (Charles River, France) were individually housed with a sexually experienced male rat for mating. A positive vaginal smear (presence of spermatozoa) defined the day 0 of gestation.

Control dams ($n = 21$) were left undisturbed throughout gestation whereas stressed dams ($n = 15$) were subjected to repeated episodes of restraint stress in a transparent cylinder (7.5 cm diameter, 19 cm long) under a bright light for 45 min three times daily (at 9:00 A.M., 12:00 P.M., and 5:00 P.M.) from day 11 of pregnancy until delivery (Maccari et al., 1995). Animals were maintained on constant temperature ($22 \pm 2\,^{\circ}C$) and 12 h light/dark cycle (light on 8:00 A.M.). 3-month-old male offspring from litters containing 10–14 pups with an equivalent number of males and females were used for the experiments. A maximum of two male pups were taken from each litter for each measure to remove any litter effects. If two pups were taken, one was included in the group treated with saline, and the other in the group treated with carbetocin. All tests took place between 9:00 A.M. and 1:00 P.M.

All experiments were approved by the institutional animal care and use committee in accordance with the principles of laboratory animal care (European communities council directive of 1986, 86/609/EEC) and following the institute for laboratory animal research "guide for care and use of laboratory animals".

2.2. Chronic carbetocin treatment

Carbetocin (1 mg/kg ≈ 1 μmol/kg, SP080756, Polypeptide group, France) or saline was chronically administrated to 3 month-old animals, i.p., 1 h before the light switch-off, following a standard protocol used for the study of antidepressant action in PRS rats (Marrocco et al., 2014). The dose and route of administration of carbetocin were selected on the basis of a previous report (Klenerova et al., 2009). In a first set of experiments rats were treated daily with saline or carbetocin for 21 days in order to start behavioral testing after 2 weeks of chronic treatment ($n = 8$–9 per group). Animals were tested for social interaction on day 15, for anxiety-like behavior on day 17, and for depressive-like behavior on days 18 and 19. On day 22, rats were killed for measurements of glutamate and GABA release ($n = 5$ per group). In a second set of experiments, PRS and control rats ($n = 8$ per group) treated daily with saline or carbetocin for 15 days were used for the measurements of plasma corticosterone levels in response to novelty stress on day 14 and sacrificed on day 16 for the measurement of mRNA levels ($n = 5$ per group) in the hippocampus, amygdala and hypothalamus.

2.3. Behavioral analysis

2.3.1. Social memory

Social memory was assessed by measuring the ability of the tested animal to recognized a juvenile challenger using a procedure adapted from Engelmann et al., 1995. Rats were individually placed in transparent cages ($39 \times 24 \times 16$ cm) for 5 min for habituation in a normally illuminated, quiet room during the light phase of the cycle (e.g., between 3:30 and 6:30 P.M.). A juvenile male rat (1 month-old) was presented to the tested adult rat for 3 consecutive sessions (5 min each). The second presentation of the juvenile occurs 30 min after the first and a third, 120 min after the first. Sessions were video-recorded and the time spent in sniffing (the tested animal sniffs the challenger's fur) and play (rearing and anterior paws interactions with the challenger) was measured by a trained observer with Observer 2.0 (Noldus, The Netherlands).