Neuropeptides xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Neuropeptides

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

Effects of chronic restraint stress on social behaviors and the number of hypothalamic oxytocin neurons in male rats

Jin Li [a,b,c], Han-Xia Li [a,b,c], Xiao-Jing Shou [a,b,c], Xin-Jie Xu [a,b,c], Tian-Jia Song [a,b,c], Song-Ping Han [a,b,c], Rong Zhang [a,b,c,*], Ji-Sheng Han [a,b,c,**]

[a] Neuroscience Research Institute, Peking University, 38 Xueyuan Road, Beijing 100191, China
[b] Department of Neurobiology, Peking University Health Science Center, 38 Xueyuan Road, Beijing 100191, China
[c] Key Lab for Neuroscience, The Ministry of Education/The Ministry of Health, 38 Xueyuan Road, Beijing 100191, China

ARTICLE INFO

Article history:
Received 9 March 2016
Received in revised form 22 July 2016
Accepted 2 August 2016
Available online xxxx

Keywords:
Chronic homotypic restraint stress
Social behavior
Oxytocin
Paraventricular nucleus

ABSTRACT

Oxytocin (OXT) and vasopressin (AVP) are considered to be related to mammalian social behavior and the regulation of stress responses. The present study investigated the effects of chronic homotypic restraint stress (CHRS) on social behaviors and anxiety, as well as its repercussions on OXT- and AVP-positive neurons in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) nuclei in rat. Male Sprague-Dawley rats receiving CHRS were exposed to repeated restraint stress of 30 min per day for 10 days. Changes in social approach behaviors were evaluated with the three-chambered social approach task. Changes in anxiety-like behaviors were evaluated in the light-dark box test. The number of neurons expressing oxytocin and/or vasopressin in PVN and SON were examined by immunohistochemistry techniques. The results demonstrated that social approach was increased and anxiety was decreased following 10-day exposure to CHRS. Furthermore, the number of OXT-immunoreactive cells in PVN was increased significantly, whereas no change in SON was seen. The number of AVP immunoreactive cells either in PVN or SON was unaffected. The results of this study suggest that certain types of stress could be effective in the treatment of social dysfunction in persons with mental disorders such as autism, social anxiety disorder. The therapeutic effects may be mediated by changes in the function of OXT neurons in PVN.

1. Introduction

The neuropeptides oxytocin (OXT) and arginine vasopressin (AVP) are evolutionarily highly involved in the regulation of various aspects of mammalian social behaviors (Lukas and Neumann, 2013; Meyer-Lindenberg et al., 2011; Harony and Wagner, 2010). Studies provide evidence that the function of these neuropeptides is impaired in mental disorders that are characterized by severe social deficits (Harony and Wagner, 2010; Caronna et al., 2008; Aspe-Sanchez et al., 2015). However, despite the neurobiological mechanisms of these disorders are largely unknown, researches in rodents and humans suggest OXT and AVP might be promising targets for such disorders.

Several lines of evidence suggest that the two peptides are crucial for regulating social behaviors impaired in autism spectrum disorders (ASD), mainly including affiliative behavior, social cognition, and social approach (Harony and Wagner, 2010; Caronna et al., 2008; Strathearn, 2009). Modahl et al. (1998) reported that plasma OXT levels were lower in children with ASD than in Control group. Gene association studies showed a link between polymorphisms in the OXT and/or AVP receptors in patients with ASD (Lauritsen et al., 2006; McCauley et al., 2005; Kim et al., 2002). There was also evidence that administration of OXT improves social cognition and reduces stereotypic movements in adults with ASD (Hollander et al., 2003). Some studies in social anxiety disorder (SAD) showed that OXT administration improved speech performance (Guastella et al., 2009) and attenuated the heightened amygdala reactivity to fearful faces in patients with SAD (Labuschagne et al., 2010).

The activity of paraventricular (PVN) and supraoptic (SON) nuclei of the anterior hypothalamus might be stimulus-dependent (Briski and

Abbreviations: OXT, oxytocin; AVP, vasopressin; HPA, hypothalamus-pituitary-adrenal; CHRS, chronic homotypic restraint stress; PVN, paraventricular nucleus; SON, supraoptic nucleus; CRH, corticotropin-releasing factor; ASD, autism spectrum disorders; SAD, social anxiety disorder.

* Correspondence to: R. Zhang, Neuroscience Research Institute, Peking University, Department of Neurobiology, School of Basic Medical Sciences, Ministry of Education, Key Laboratory for Neuroscience, Ministry of Health, 38 Xueyuan Road, Beijing 100191, PR China.
** Correspondence to: J.-S. Han, Neuroscience Research Institute, Department of Neurobiology, Peking University Health Science Center, Key Laboratory of Neuroscience, The Ministry of Education and Ministry of Public Health, 38 Xueyuan Road, Beijing 100191, PR China.
E-mail addresses: zhangrong@bjmu.edu.cn (R. Zhang), hanjisheng@bjmu.edu.cn (J.-S. Han).

http://dx.doi.org/10.1016/j.npep.2016.08.011
0143-4179/

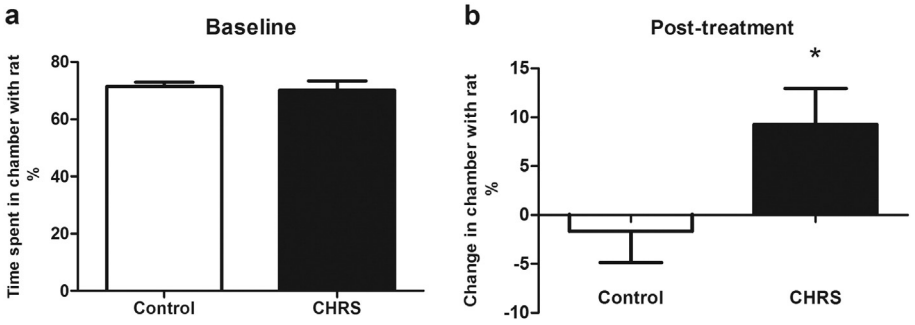

Fig. 1. Effects of CHRS on social approach in male adult rats.

Gillen, 2001; Palkovits, 2000). Previous studies showed that PVN and SON are activated by osmotic and reproductive stimuli as well as during stress (Engelmann and Ludwig, 2004; Wotjak et al., 1998; Engelmann et al., 1999). OXT and AVP are nonapeptides synthesized in PVN and SON of the hypothalamus (Sofroniew, 1983). Besides being involved in regulating mammalian social behaviors, they also play an important role in the regulation of stress response (Nishitani et al., 2004; Pirnik et al., 2004; Pirnik and Kiss, 2005; Wang et al., 2009).

OXT attenuates stress-induced activation of the hypothalamus-pituitary-adrenal (HPA) axis, thus modulates stress response in rodents (Windle et al., 1997; Neumann, 2002). Stress exposure potenciates OXT secretion into the peripheral circulation (Lang et al., 1983; Kasting, 1988) and within the brain as reflected by increased OXT concentration in the cerebrospinal fluid (Ivanyi et al., 1991). It has also been reported that OXT is released within the hypothalamus in response to shaker (Nishioka et al., 1998) and forced swimming (Wotjak et al., 1998) stress in rat. Study of oncogene expression has demonstrated that restraint stress activates oxytocinergic neurons in PVN and SON (Miyata et al., 1995). OXT administration also attenuates the increase in gene expression of corticotropin-releasing factor (CRH) in PVN in response to acute restraint stress in rats (Windle et al., 2004). On the other hand, repeated experiences with the same stressor may produce habituation or reduction of behavioral responses (Zheng et al., 2010; Yoshimoto et al., 2012).

In contrast to OXT, fewer studies have been carried out to investigate the role of AVP in stress responses. AVP acts as an important modulator of adrenocorticotropic hormone release in response to stress by potentiating the effect of CRH (Antoni, 1993). Acute stress induces rapid and concomitant release of CRH and AVP into the pituitary portal circulation from parvocellular neurons of PVN (Kovacs and Sawchenko, 1996). Immunohistochemical studies have shown that following repeated immobilization stress CRH stores remain unchanged, but there are progressive increases in AVP stores as well as the number of CRH nerve endings containing AVP (de Goeij et al., 1991).

Other studies have shown that exposure to chronic stress increases OXT content in PVN in rat (Zheng et al., 2010; Yoshimoto et al., 2012). Exposure to repeated immobilization also increased oxytocin mRNA levels in the hypothalamus (Zheng et al., 2010; Babygirija et al., 2010). However, relatively few studies have directly examined the repeated restraint stress on social behaviors.

The present studies were therefore designed to investigate the effects of chronic homotypic restraint stress (CHRS) on social behaviors (social approach and anxiety) of male rats. It has been well accepted that the abnormal social behavior and excessive anxious reactions are the core symptoms of mental disorders like ASD and SAD. It is not clear how stress therapy helps to improve the abnormal behavior in the symptoms of such disorders. OXT and/or AVP neurons in hypothalamus have been shown to be involved in mediating stress responses and social behavior. In addition, these neurons have also been suggested to be at least partially responsible for the etiology of autism. We hypothesized that the therapeutic effect of chronic stress is mediated by changes in the function of hypothalamic OXT and/or AVP neurons.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley (SD) rats weighing 220–250 g, aged 65–85 d at the beginning of the experiment were obtained from experimental animal department of Peking University Health Science Center. They were housed in a 12:12 h light/dark cycle (lights on at 07:00 h) with food and water ad lib. The room temperature was maintained at 24 ± 1 °C and relative humidity at 50%. Twenty rats were evenly divided into two groups: the CHRS group and the Control group. Animals were housed 4 to 5 per cage (590 mm × 380 mm × 200 mm). In order to reduce the stress due to the novel environment, animals were individually acclimated for 5 min/two times daily for 2 days on the day before the experiment. Then we performed a baseline behavioral testing. The

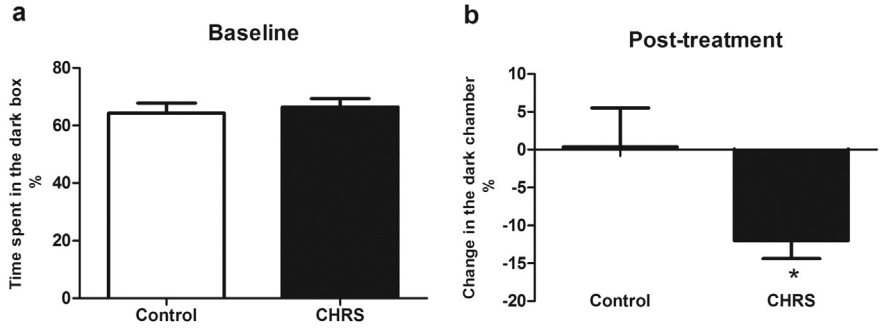

Fig. 2. Effects of CHRS on anxiety level in male adult rats. The percent of time spent in dark box reflects anxiety level, CHRS reduced anxiety behavior in the light-dark box. Data represent mean ± SEM (n = 10). *$P = 0.042$, an unpaired *t*-test was used to compare changes in anxiety-like behaviors (con vs stressed) in Panel b.

Please cite this article as: Li, J., et al., Effects of chronic restraint stress on social behaviors and the number of hypothalamic oxytocin neurons in male rats, Neuropeptides (2016), http://dx.doi.org/10.1016/j.npep.2016.08.011