Psychoneuroendocrinology (2013) 38, 1397—1406

Available online at www.sciencedirect.com

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Neonatal neurosteroid levels are determinant in shaping adult prepulse inhibition response to hippocampal allopregnanolone in rats

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Received 26 July 2012; received in revised form 5 December 2012; accepted 11 December 2012

KEYWORDS
Neurodevelopment;
Hippocampus;
Behaviour;
Allopregnanolone;
Pregnenolone sulphate;
Prepulse inhibition;
Acoustic startle response;
Schizophrenia;
Rat

Summary Diverse studies indicate that the alteration of the physiological levels of neurosteroids in early neonatal phases provokes alterations in the maturation of certain cerebral structures. Allopregnanolone (ALLO) has important modulatory effects in the hippocampus during the postnatal period where the adult pattern of inhibitory transmission is being established. In order to study whether endogenous neonatal ALLO levels would be a determinant parameter involved in mediating adult hippocampal GABAA system maturation, we investigated the effects of neonatal finasteride (50 mg/kg, SC) treatment and ALLO (ALLO; 20 mg/kg, SC) supplementation on an animal behavioural model with relevance to neurodevelopmental disorder, such as schizophrenia. Two sets of experiments were conducted. Neonatal treatment (from postnatal day (pnd) 5 to pnd9) was performed in 23 male Wistar rats and steroid quantification was performed in hippocampal homogenates at pnd9. A second group (n = 127) underwent neonatal treatment (pnd5—pnd9) and were submitted to hippocampal surgery at 80 d. The behavioural response to bilateral intrahippocampal neurosteroid administration (ALLO, 0.2 μg/0.5 μl per side or pregnenolone sulphate 5 ng/0.5 μl per side) on novelty-induced exploration activity and prepulse inhibition (PPI) was assessed at 95 d. Results showed that neonatal ALLO and finasteride administration decreased novelty directed exploratory behaviour and impaired the prepulse inhibition of the acoustic startle response at 95 days of age. Moreover, intrahippocampal ALLO increased head-dipping behaviour independently of the neonatal treatment, while intrahippocampal ALLO decreased PPI only in finasteride and ALLO groups. The results obtained in the present study indicate the importance of neonatal neurosteroid levels in the development of hippocampal function and their relevance in a behavioural phenotype that some have likened to

1. Introduction

Neurosteroids, typified by the progesterone metabolite allopregnanolone (ALLO), potently modulate neuronal

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0306-4530/$ — see front matter
http://dx.doi.org/10.1016/j.psyneuen.2012.12.007

excitability through endocrine, paracrine or autocrine actions at GABAA receptors (Belelli and Lambert, 2005). Under physiological conditions, neurons are exposed to neurosteroids but also to changes in neurosteroid levels related to stress, pregnancy, menstrual cycle or menopause (Frye, 2009). In the last few years the important role that the GABAA neurosteroids modulators play in the development of the central nervous system (CNS) throughout life has been revealed, especially in late pregnancy, early infancy and adolescence (Mellon, 2007). Diverse studies indicate that the alteration of the physiological levels of neurosteroids in early neonatal phases provokes alterations in the maturation of certain cerebral structures such as the hippocampus (Cooper et al., 1999; Mtchedlishvili et al., 2003), the GABAergic thalamic-cortical system (Grobin et al., 2003; Gizerian et al., 2004) or the meso-cortical and meso-striatal dopaminergic systems (Muneoka and Takigawa, 2002; Gizerian et al., 2006). ALLO has important modulatory effects in the hippocampus during the postnatal period where the adult pattern of inhibitory transmission is being established (postnatal day 9 (pnd9) − pnd12 period) (Cooper et al., 1999). Changes in ALLO biosynthesis during development and in early neonatal period could affect GABAA receptor subunit expression in the hippocampus. For instance, hippocampal GABAA receptors have increased sensitivity to ALLO during postnatal development, which may be related to increased expression of the alpha1 subunit (Mtchedlishvili et al., 2003). Moreover, recent studies indicate that neurosteroids can participate in the maturation of the hippocampus not only in neonatal but also in gestational periods, such as finasteride treatment, an inhibitor of ALLO synthesis (Azzolina et al., 1997; Mukai et al., 2008), increased both activated caspase-3 and pyknotic staining of mature neurons, and there was also increased apoptotic cell death of astrocytes in the CA1 and CA3 regions of the hippocampus (Yawno et al., 2009).

Recent experiments carried out in our laboratory have shown that sub-chronic neonatal administration (from the fifth to the ninth day of life) of finasteride increases emotional reactivity in situations of stress or conflict in the adolescent age, as reflected by the reduction in exploration of a novelty situation (decrease in novelty-directed activity and head-dips (Darbra and Pallarès, 2010). Acute neonatal (fifth post-natal day, pnd5) administration of ALLO increases novelty-directed locomotion in adulthood measured in the open field and decreases the anxiolytic effects of the benzodiazepine lorazepam measured in the elevated plus maze test (Darbra and Pallarès, 2009). Also, sub-chronic neonatal ALLO administration (from the fifth to the ninth day of life) deteriorates PPI in adulthood (Darbra and Pallarès, 2010). It is important to note that neurosteroids have been found in higher concentrations in adult hippocampus of humans and rodents than in other brain structures (Vallée et al., 1997; Concas et al., 1998; Weill-Engerer et al., 2002). The hippocampus seems to be important in mediating the promnesic (Vallée et al., 1997) and anticonvulsive (Martin-Garcia and Pallares, 2005) profiles of the neurosteroid pregnenolone sulphate (PregS) in adulthood. Moreover, the hippocampus is also directly involved in untrained anxiety (Engin and Treit, 2007) and environmental novelty reactions in animals (Jeewajee et al., 2008) and PPI seems to depend on hippocampal activity (Howland et al., 2004).

In the present study, we investigated the effects of neonatal finasteride treatment and ALLO supplementation on an animal behavioural model with relevance to neurodevelopmental disorder, such as schizophrenia. Because our previous results showed that the alteration of the physiological neonatal ALLO levels, by means of ALLO or finasteride administration, can alter the normal adult responses to novelty measured in the Boissier test (Darbra and Pallarès, 2009) and impairs PPI in adulthood (Darbra and Pallarès, 2010), we hypothesized that neonatal ALLO levels would be involved on the maturation of inhibitory hippocampal system and related functions, i.e. exposure to a novel environment and the achievement of the prepulse inhibition. Consequently, the behavioural response to intrahippocampal neurosteroids administration would be different depending on neonatal ALLO levels. PPI represents an index of sensorimotor gating, which thought to be governed by central inhibitory mechanisms, and its deterioration is present in several neuropsychiatric disorders, including schizophrenia (Swerdlow et al., 2006). Hyperresponsiveness to stress and novelty are also classically observed in schizophrenic patients.

Because pharmacological studies do not always reflect physiological effect of endogenous compound, measuring neurosteroid levels following neonatal ALLO and finasteride treatments is a prerequisite to investigate a role of neonatal ALLO in behavioural alterations. Hippocampal neonatal neurosteroids were measured using a specific, sensitive, and accurate steroid quantification method based on isotope dilution combined with gas chromatography/mass spectrometry (Vallée et al., 2000). Accurate assessment of neurosteroid levels in small brain regions is still a challenge, and few data are currently available from studies investigating alterations in neurosteroid levels in individual brain areas with highly sensitive and specific analytic methods (George et al., 2010).

2. Methods

2.1. Animals

The subjects were 127 male Wistar rats derived from twelve pairings raised in an in-house colony (Laboratori de Psicobiologia, Universitat Autònoma de Barcelona) and allowed food and water ad libitum. The rats were housed in a temperature-controlled animal room (22−24 °C) on a 12-h light/dark cycle. Experimental sessions were run during the light portion of the cycle (lights on at 08:00 h). The male breeders were separated from the females after 48 h. Pregnant females were closely monitored and on the day of birth (called day 0), mothers were removed from the cage and litters were culled to 10 pups (with a maximum 7 and a minimum of 3 males). In order to avoid any cohort effects, each litter of the same colony was assigned to different neonatal treatment groups (ALLO, finasteride, vehicle solution or non-handle, NH). After weaning (postnatal day (pnd) 21), males were separated into groups of brothers (with a maximum of five subjects per cage), and females were sacrificed. No visible animal health alterations were detected during development as a consequence of the neonatal treatments. No differences in body weight among groups were observed at pnd30. For hippocampal