Hormones and Behavior 65 (2014) 142–153

Contents lists available at ScienceDirect

Hormones and Behavior

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

Neonatal exposure to estradiol decreases hypothalamic allopregnanolone concentrations and alters agonistic and sexual but not affective behavior in adult female rats

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ARTICLE INFO

Article history:
Received 23 August 2013
Revised 3 December 2013
Accepted 15 December 2013
Available online 22 December 2013

Keywords:
Neonatal estradiol
Allopregnanolone
Hypothalamus
Affect
Agonistic behavior
Sexual behavior
Social learning

ABSTRACT

Exposure of developing female rats to estradiol during the perinatal period induced long-lasting dysregulation of gonadal axis and decreased cerebrocortical and plasma concentrations of allopregnanolone. We have now examined the effects of neonatal estradiol administration in female rats on hypothalamic allopregnanolone concentrations and on exploratory, affective, agonistic and sexual behaviors as well as social learning. A single administration of β-estradiol 3-benzoate (EB, 10 μg) on the day of birth resulted in a delay of vaginal opening, acyclicity and ovarian failure. These alterations were associated with a significant decrease in the concentrations of allopregnanolone in the hypothalamus at 21 and 60 days, but not at 7 days, after birth. Neonatal administration of EB also increased agonistic behaviors in adult rats, such as dominant behaviors and following of an ovariectomized intruder, while living attacks unaffected. EB-treated rats showed also an increase in anogenital investigation, associated with a drastic reduction in spontaneous and induced female sexual behaviors (receptivity and proceptivity). In contrast, neonatal administration of EB did not affect locomotor activity, anxiety- and mood-related behaviors, the social transmission of flavor preferences, and seizures sensitivity. These effects of estradiol suggest that it plays a major role in regulation of both the abundance of allopregnanolone and the expression of agonistic and sexual behaviors, while failing to influence affective behaviors and social learning. Thus, the pronounced and persistent decrease in hypothalamic allopregnanolone concentration may be related to the manifestation of agonistic and sexual behaviors.

Introduction

The 3α,5α-reduced metabolite of progesterone, allopregnanolone, is a neurosteroid that exerts a rapid change in neuronal excitability and elicits behavioral effects within seconds to minutes from its administration to experimental animals (Biggio and Purdy, 2001; Wang, 2011). The understanding of the physiological role of this neurosteroid was greatly improved by the finding that allopregnanolone is the most potent and efficacious positive allosteric modulator of gamma-aminobutyric acid type A ($GABA_A$) receptor function (Majewska, 1992; Wang, 2011). In fact, animal studies have shown that allopregnanolone, administered systemically or intracerebroventricularly, induces marked anxiolytic, sedative–hypnotic, antidepressant, and anticonvulsant effects, similar to those induced by classical positive modulators of $GABA_A$ receptors, such as benzodiazepines (Biggio and Purdy, 2001). Moreover, allopregnanolone facilitates social and sexual behaviors (Frye, 2001) and exhibits analgesic and neuroprotective actions (Djebaili et al., 2005; Mensah-Nyagan et al., 2008). On the other hand, a number of reports have indicated that allopregnanolone may also have some non-beneficial effects. Thus, allopregnanolone induces irritability/aggression (Fish et al., 2002; Miczek et al., 1997), and a detrimental learning profile when injected systemically (Johansson et al., 2002) or directly into the brain (Mayo et al., 1993).

Given that allopregnanolone is synthesized both in the periphery and in the brain from endogenous progesterone (Mellon and Griffin, 2002), physiologic or pharmacologically-induced fluctuations in the concentration of this gonadal steroid are paralleled by changes in the synaptic concentration of allopregnanolone, which contribute to the regulation of $GABA_A$ receptor activity. As $GABA_A$ receptors are implicated in a variety of neuropsychophysiologic phenomena, including anxiety, sleep, seizures, depression, and social and sexual behaviors, such fluctuations in the concentrations of this neurosteroid may contribute to the cognitive and psychiatric manifestations of conditions characterized by marked changes in the hormonal milieu.

Several findings indicate that allopregnanolone plays an important role during brain development (Mameli et al., 2005). In fact, neonatal allopregnanolone promotes the establishment of neuronal circuitry and supports the survival of developing neurons (Griffin et al., 2004).

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http://dx.doi.org/10.1016/j.yhbeh.2013.12.009

Moreover, alterations in neonatal allopregnanolone levels have a profound effect on the morphology and the structure of several brain areas such as the cortex and the thalamus (Grobin and Morrow, 2001; Grobin et al., 2003, 2006). Accordingly, alterations in adult behavior have also been reported. Thus, manipulation of neonatal allopregnanolone levels alters performance in the elevated plus maze test and aversive learning in the passive avoidance test (Martin-Garcia et al., 2008). In addition, allopregnanolone administration from postnatal day 5 to postnatal day 9 has been shown to alter responses to $GABA_A$ modulators (such as benzodiazepines) during adulthood (Darbra and Pallares, 2009), and to induce an anxiolytic profile in the elevated plus maze test (Darbra and Pallares, 2012). Taken together, these evidences suggest that alterations in allopregnanolone concentrations during development might have important consequences for adult behavior.

Gonadal steroids, in particular androgens and estradiol, play important roles in the regulation of sexual dimorphism as well as in the growth and development of the nervous system during both prenatal and perinatal periods (McCarthy, 2008). Abnormal levels of sex hormones in the perinatal period can induce profound alterations in neuroendocrine activity, which influence both reproductive function and hormonal levels in adulthood (Foecking et al., 2005; McCarthy, 2008). Treatment of female neonates with testosterone or estrogen on postnatal days 1–10 renders them permanently incapable of undergoing the luteinizing hormone (LH) surge (Korenbrot et al., 1975), and consequently results in marked perturbations of the hormonal milieu in adulthood (Handa et al., 1985; Rodriguez et al., 1993). Recently, we have shown that neonatal administration of EB to female rats resulted in marked decreases in the cerebrocortical and plasma concentrations of allopregnanolone that persisted into adulthood (Calza et al., 2010). These effects were accompanied by changes both in the expression of specific $GABA_A$ receptor subunits in the cerebral cortex as well as in the behavioral sensitivity to diazepam. We have now evaluated whether neonatal administration of EB might influence allopregnanolone concentrations in the hypothalamus of infant (7 days), juvenile (21 days) and adult (60 days) female rats. Moreover, given that allopregnanolone is involved in anxiolytic-like, agonistic, social and reproductive behaviors, we also examined whether neonatal administration of EB affects different experimental paradigms most commonly used for the analysis of exploratory, emotional, social, learning, and sexual behaviors.

Methods

Animals

Female Sprague–Dawley rats (Charles River, Calco, Italy) were bred in our colony and maintained on an artificial 12-h-light, 12-h-dark cycle (light on from 08:00 to 20:00 h) at a constant temperature of 22 ± 2 °C and a relative humidity of 65%. Food and water were available ad libitum. Adequate measures were taken to minimize pain or discomfort of animals whose care and handling throughout the experimental procedures were in accordance with the European Parliament and the Council Directive of 22 September 2010 (2010/63/EU) and were approved by the local ethics committee.

Hormone treatments

On the day of birth (day 0), male pups were removed from the litter while female pups were injected subcutaneously (s.c.) with 10 μg of EB (Sigma-Aldrich, Milan, Italy) in 50 μl of sesame oil (Sigma-Aldrich, Milan, Italy) or with 50 μl of sesame oil (vehicle) (Rodriguez et al., 1993; Solum and Handa, 2002). To avoid leakage of EB or oil due to body movements, all pups were injected under hypothermic anesthesia (Solum and Handa, 2002). After injection, neonatal EB- or vehicle-treated female pups were allowed to recover near a heating lamp before being randomly distributed among litters of the same age so that each mother had five to eight pups. All female pups within a litter received the same treatment. Immediately after weaning, neonatally treated female rats were housed in groups of six to eight per cage: each cage consisted of only vehicle- or only EB-treated rats. They were sacrificed by decapitation at 7 (infant), 21 (juvenile) or 60 (adult) days after birth for measurement of hypothalamic allopregnanolone levels. Behavioral tests took place between days 60 and 110. To avoid effects of estrous cycle status and the stress of vaginal smears on steroid concentrations and behavior, vehicle-treated animals were assessed in randomized phases of the estrous cycle. On day 60, vehicle- and EB-treated rats that underwent the induced sexual behavior test and naïve rats used as demonstrators in the social learning test or as intruders in the resident–intruder test, were ovariectomized. Bilateral ovariectomies were performed under Equithesin anesthesia [pentobarbital 0.81 g, chloral hydrate 4.25 g, magnesium sulfate 2.13 g, ethanol 100% 11.6 ml and propylene glycol 42.8 ml in distilled water to a final volume of 100 ml, administered intraperitoneally (i.p.), 3 ml/kg], 3–4 weeks prior to the behavioral tests. The vaginal cytology of the ovariectomized rats confirmed that they were not cycling.

Measurement of vaginal opening and vaginal smear checks

Female pups were checked daily for vaginal opening. Vaginal smears were performed from vaginal opening until day 60. A female rat which showed a constant 4- or 5-day vaginal estrous cycle was regarded as an animal with a regular estrous cycle. When a vaginal smear contained cornified cells throughout the examination term, it was considered as persistent estrous, even if a few leukocytes were occasionally observed. In those few cases in which a diestrus slide was occasionally observed (once or twice over the observation period), the rat would still be assigned to the "persistent estrous" group. 10 rats from each experimental group (neonatally EB- and vehicle-treated) were examined; rats were randomly selected from 3 different cages for each experimental group.

Hematoxylin and eosin staining of ovarian sections

On day 60, one set of neonatally vehicle- or EB-treated female rats were ovariectomized and the ovaries were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) through three consecutive steps: the first two steps were performed at room temperature for 1 h each, the last step was performed at 4 °C overnight. The tissue was then rinsed in PBS, dehydrated and embedded in paraffin. Ovarian tissue sections of 10 μm were prepared for hematoxylin and eosin staining. Tissue sections of ovaries were deparaffinized in xylene, dehydrated through an ethanol series of 50, 70, 80, 90 and 100%, and stained with hematoxylin and eosin. After dehydration and clearing with fresh xylene, sections were mounted with Canada balsam and observed on an inverted microscope in brightfield (ZEISS, Axio Observer Z.1). The images were acquired with a high resolution camera (ZEISS Axiocam MR-m) and were analyzed by Axiovision software for the presence of corpora lutea (CL) and Graafian follicles (GF). 9 rats from each experimental group (neonatally EB- and vehicle-treated) were examined; rats were randomly selected from 3 different cages for each experimental group.

Steroid extraction and assay

For measurement of hypothalamic allopregnanolone concentrations, female rats were neonatally treated with EB or vehicle and were sacrificed by decapitation at 7, 21 and 60 days after birth. 10 rats from each experimental group were used; rats were randomly selected from 3 different cages for each experimental group. The brain was rapidly (<1 min) removed, and the hypothalamus was dissected on ice and frozen at −80 °C until steroid extraction. Allopregnanolone present in hypothalamic homogenates (10 mg of tissue in 100 μl of phosphate-