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The Nhlh2 transcription factor is required for female sexual behavior and reproductive longevity

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Abstract

Nhlh2 is a member of the basic helix-loop-helix (bHLH) transcription factor family and is expressed in developing and adult neuroendocrine tissues such as the pituitary and hypothalamus. Targeted deletion of Nhlh2 (N2KO) in mice results in hypogonadism and obesity. While gonadally intact male N2KO mice are infertile and lack male sexual behavior, female N2KO mice can become pregnant and carry litters to full term. Unlike normal females in which fertility averages 8–12 months with approximately one pregnancy per month, N2KO females have a shorter reproductive span with most females supporting only three to four pregnancies in a 9-month period. In addition, N2KO females exhibit abnormal estrous cycles characterized by a truncated estrus and a prolonged proestrus. We have found that while young female N2KO mice ovulate the same number of oocytes as normal females in response to exogenous hormones, the number of oocytes released by aged N2KO females is reduced over 50%. Interestingly, oocytes from N2KO females are equally competent for in vitro fertilization assays when compared to oocytes from similarly aged normal and heterozygous mice. We have further demonstrated that both young and old N2KO females show at least a 50% reduction in hormone-stimulated sexual behavior as measured by their lordosis quotient. This suggests that N2KO females show a lifelong behavioral hyporesponsiveness to exogenous steroid hormones accompanied by a reduction in reproductive longevity via reduced ovulation with aging. Potential gene regulatory mechanisms that involve the action of the Nhlh2 transcription factor on female fertility and sexual behavior are discussed.

Keywords: Sexual receptivity; Lordosis; In vitro fertilization; Estrous cycle; Premature reproductive senescence; bHLH transcription factor; Mouse/mice; Fertility

Introduction

At a minimum, successful reproduction requires that a female produce functional oocytes and demonstrate mating behavior in response to the male. If either of these are lacking, fertility of the animal will be reduced. It is well established that estrogen (E) and progesterone (P) are required for both sexual behavior and ovulation (Etgen et al., 1999; Pfaff et al., 2002). In addition, gonadotropin hormone releasing hormone (GnRH) must be released into the hypophyseal portal to trigger production and release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) into the circulation. In female mammals, these hormones are part of a regulatory loop in which FSH stimulates follicular growth as well as production of E, which feeds back to the hypothalamus, providing a positive signal that results in the LH surge. This, in turn, results in ovulation and production of P by the corpus luteum. In the mouse, the entire estrous cycle takes approximately 5 days with animals spending the most time in the diestrus and estrus phases of the cycle (Rugh, 1990).

In normal, intact females, sexual behavior is elicited during the estrus phase of the cycle when P levels are high, which follows high E levels in the late pro- and early estrus phases. During estrus, introduction of a male leads to a hormonally dependent, reflexive display of copulatory behavior called lordosis, which is characterized by an arching of the back and a rigid stance that facilitates male copulation.

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Ovariectomy obliterates lordosis by removing the sources of E and P, but treatment of gonadectomized mice sequentially with E and P restores this receptive behavior. Receptors for E and P are produced in the hypothalamus and medial preoptic area (MPOA) and an extensive amount of research supports their role in genomic regulation of female reproductive behavior and fertility. For example, mice with a deletion of the alpha form of the estrogen receptor (ERαKO) display no lordosis response when either gonadally intact (Ogawa et al., 1996) or when ovariectomized and administered steroids (Ogawa et al., 1998; Rissman et al., 1997). Furthermore, when stud males are placed in a cage with intact ERαKO females in diestrus, these females are attacked more often and mounted less than normal females (Ogawa et al., 1996). In contrast, females with a deletion of the beta form of the estrogen receptor show normal sexual receptivity (Ogawa et al., 1999). Deletion of both the A and B forms of the progesterone receptor in mice (PRKO mice) ameliorates female sexual behaviors (Lydon et al., 1995, 1996).

In addition to the MPOA, neurons in the ventromedial hypothalamus (VMH) are necessary for female sexual behavior and reproduction. Rodents with VMH lesions are not receptive to males (Pfaff and Sakuma, 1979) while electrical stimulation of the VMH can result in lordosis behavior with exogenous hormone treatment. Furthermore, implantation of fetal preoptic area explants into the third ventricle, adjacent to the VMH, results in resumption of lordosis in mice carrying a mutation of the GnRH gene (Gibson et al., 1987), as does injection of estrogens into this area in ovariectomized rats (Butera and Beikirch, 1989). Receptors for both E and P are present in the VMH (Garris et al., 1983; Koch and Ehret, 1989).

Female fertility and sexual receptivity requires the activation of gene regulatory pathways that are dependent on the transcriptional activation of target genes by the estrogen and progesterone receptors. The mouse ER (ERα and ERβ) and PR (A and B forms) are members of the steroid receptor superfamily of transcription factors (Schott et al., 1991; White et al., 1987), and bind to DNA sequences (i.e., estrogen and progesterone response elements) that are found in the promoters of target genes. Ligand-activated receptors can also interact with and modulate other transcription factors and co-activators (Kushner et al., 2000). Many estrogen and progesterone-responsive neuronal genes have been identified and many of these are required for female fertility (Frohlich et al., 2002).

In this paper, we describe our studies on female reproduction and reproductive behavior in the Nhlh2 knockout mouse line (N2KO). Nhlh2 is a member of the basic helix-loop-helix domain (bHLH) family of transcription factors. This family of transcription factors binds as homo- and heterodimers to an E-box motif in the promoter region of genes. While the E-box motif for Nhlh2 has not been identified, that for a homologous bHLH transcription factor, Nhlh1 is CAGCTG (Brown and Baer, 1994). Nhlh2 is expressed throughout the developing central and peripheral nervous system from day 8.5 of embryogenesis in mice with high expression in the developing hypothalamus and pituitary. In the adult, Nhlh2 is expressed in the anterior lobe of the pituitary and in the adult hypothalamus (Good et al., 1997). Both male and female N2KO mice are hypogonadal with an approximate 50% reduction or more in gonad size and rudimentary secondary sexual organs (seminal vesicles and preputial glands) in males. Females have threadlike uteri and a delay in vaginal opening. While intact male N2KO mice are completely infertile because of a lack of male sexual behavior (Good et al., 1997), some female N2KO mice can become pregnant and carry litters to full term. This seemingly paradoxical result appears to be caused by the ability of male pheromones to facilitate uterine and pubertal development of female N2KO mice (Cogliati, Good and Kirsch, unpublished data; Good et al., 1997). In normal female mice, fertility averages an 8- to 12-month reproductive longevity (Rugh, 1990) with approximately one pregnancy per month. We will demonstrate in this paper that although some N2KO females can produce offspring, the number of pregnancies over their lifetime is reduced, and that N2KO female mice show a lifelong reduction in hormone-induced lordosis behavior and reduced ovulation with aging. Furthermore, these are the first data to identify a neuronal transcription factor, Nhlh2, which is required for the display of both male and female sexual behavior and gametogenesis. Finally, we propose potential targets of Nhlh2 in the neuronal regulation of fertility.

Methods

Animals

All work involving animals was done in compliance with the Institutional Animal Care and Use Committee at the University of Massachusetts, Amherst. Heterozygous breeders between 2 months and 1 year of age were used for maintenance of the Nhlh2 line of mice. For heterozygous crosses, the expected 1:2:1 ratio of normal to heterozygous to knockout animals, with a 1:1 ratio of males to females was obtained. At 3 weeks of age, all pups were weaned into new cages by sex, and given a unique four-digit ear tag. At the same time, a 1-cm tail biopsy was taken and immediately placed on dry ice for DNA preparation and genotype analysis. N2KO and normal mice were maintained in 12-h photoperiod (lights on at 07:00) with ad lib food (4.5% crude fat). For breeding analysis, females were caged with normal males or Nhlh2 heterozygous (HET) males. Females had a starting age of 8–12 weeks and the total number of pregnancies was counted over a 9-month period.

Estrous cycles

All females were group housed and in a room that also contained male mice. Females were analyzed for vaginal cytology between 8 and 10 AM every morning. To do this,