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NEUROPEPTIDE Y SIGNALING IN THE DORSAL RAPHE NUCLEUS INHIBITS MALE SEXUAL BEHAVIOR IN MICE

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Abstract—Animals change their biological activities depending on their nutritional state. Reproductive functions, including sexual behavior, are suppressed under low-energy conditions; however, the underlying neuronal mechanism is poorly understood. Neuropeptide Y (NPY) is an orexigenic molecule released in response to low-energy conditions and has an inhibitory effect on sexual behavior. We examined how NPY is involved in energy state-dependent regulation of male sexual behavior. Mounting, intromission, and ejaculation were evaluated as parameters of sexual behavior. Almost all parameters indicated that fasting for 24 h suppressed male sexual behavior. Intracerebroventricular injection of NPY inhibited sexual behavior in males that free-fed for 8 h following 24-h fasting (fed males). We next examined whether the dorsal raphe nucleus (DRN), in which serotonergic (5-HT) neurons are distributed, is involved in NPY-mediated inhibition of male sexual behavior. NPY-positive processes immunoreactive for a presynaptic marker, synaptophysin, were distributed in the DRN of both fed and fasted males. Expression of the NPY Y1 receptor in 5-HT neurons was also observed. Direct injection of NPY or 8-OH-DPAT (a 5-HT_{1A} receptor agonist that inhibits the activity of 5-HT neurons) into the DRN inhibited male sexual behavior in fed males. In contrast, injection of BIBP-3226, a NPY Y1 receptor antagonist, or (+)-DOI hydrochloride (DOI), a 5-$HT_{2A/2C}$ receptor agonist that activates 5-HT neurons, into the DRN partially recovered male sexual behavior in 24-h fasted males. These results suggest that NPY inhibits serotonergic neuronal activity via the Y1 receptor in the DRN, resulting in suppression of male sexual behavior in low-energy conditions.

Key words: male sexual behavior, neuropeptide Y, dorsal raphe nucleus, serotonin.

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Abbreviations: 5-HT, serotonin or 5-hyrodxytryptamine; 8-OH-DPAT, (±)-8-hydroxy-2-dipropylaminotetralin; DOI, (+)-DOI hydrochloride; DRN, dorsal raphe nucleus; MPOA, medial preoptic area; MRN, median raphe nucleus; NPY, neuropeptide Y; PBS, phosphate-buffered saline; TPBS, Triton X-100 in PBS; TPH, tryptophan hydroxylase; VTA, ventral tegmental area.

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INTRODUCTION

Nutritional state significantly affects biological activities in animals, from the cellular to the whole-body level. Although changes in nutritional state are directly involved in the survival of the individual, they also affect reproductive functions. For example, the release of gonadotropin-releasing hormone in the hypothalamus is inhibited under low-energy conditions (Wahab et al., 2013), resulting in the suppression of spermatogenesis and ovulation (Bronson, 1990). Nutritional state also affects reproductive functions at the behavioral level. Sexual behavior is inhibited under low-energy conditions in Syrian hamsters and meadow voles (Klingerman et al., 2011; Sabau and Ferkin, 2013). Thus, suppression of reproductive function is important for ensuring preferential energy allocation for the survival of the individual.

It remains unknown which molecules mediate energy state-dependent regulation of reproductive functions. In the central nervous system, orexigenic neuropeptides such as neuropeptide Y (NPY) and agouti-related protein are secreted under food-restricted conditions (Stark et al., 2013). Although these substances primarily act to induce feeding behavior (Sobrino et al., 2014), they also affect gonadal functions by regulating the release of gonadotropin-releasing hormone (Wahab et al., 2013). Moreover, orexigenic molecules also regulate sexual behavior. NPY and orexin have an inhibitory and facilitative effect on male sexual behavior in rats, respectively (Clark et al., 1985; Ammar et al., 2000; Muschamp et al., 2007; Di Sebastiano et al., 2010). However, the effect of orexigenic molecules in nutritional state-dependent regulation of sexual behavior has not been clarified.

NPY is a well-known orexigenic neuropeptide that is abundantly expressed in the hypothalamus. NPY neurons in the arcuate nucleus project to the paraventricular nucleus and induce feeding behavior (Stark et al., 2013). Moreover, NPY is expressed in a variety of brain regions, including the amygdala, frontal cortex, hippocampus, and nucleus accumbens (Adrian et al., 1983; Allen et al., 1983) and is involved in multiple physiological functions including addiction, anxiety, circadian rhythm, learning, and thermoregulation (Heilig, 2004; Bi, 2013; Borbély et al., 2013; Morin, 2013; Gonçalves et al., 2015). These diverse functions are mediated by five $G_{i/o}$-protein-coupled receptors (Y1, Y2, Y4, Y5, and Y6) (Michel et al., 1998). We previously reported that NPY signaling via the Y1 receptor in the dorsal raphe nucleus

(DRN) mediates the inhibition of maternal behavior under low-energy conditions in mice (Muroi and Ishii, 2015). Some NPY neurons in the arcuate nucleus project into the DRN (Yoon et al., 2013). Moreover, NPY has an inhibitory effect on male sexual behavior in rats (Clark et al., 1985; Ammar et al., 2000). Therefore, we hypothesized that NPY signaling via the Y1 receptor in the DRN also mediates the inhibition of male sexual behavior under low-energy conditions.

The DRN is located in the midbrain and contains a large population of serotonin (5-hyrodxytryptamine; 5-HT) neurons, which project their axons to a variety of brain regions (Michelsen et al., 2007). Serotonin has also been implicated in numerous functions including anxiety, depression, panic disorder (Paul and Lowry, 2013; Paul et al., 2014), food intake (Bello and Liang, 2011), and male sexual behavior (Rubio-Casillas et al., 2015). Moreover, serotonergic neurons in the DRN express the Y1 receptor in mice (Muroi and Ishii, 2015). Therefore, NPY-mediated modulation of 5-HT neuronal activity in the DRN may be also involved in energy state-dependent regulation of male sexual behavior. In the present study, we examined whether NPY signaling via the Y1 receptor in the DRN regulates 5-HT neuronal activity to inhibit male sexual behavior under low-energy conditions.

EXPERIMENTAL PROCEDURES

Animals

Sexually naïve *ddY* male mice (N = 83, 8–12 weeks old; SLC, Hamamatsu, Japan) were housed in shredded paper bedding with a pellet diet (Clea, Tokyo, Japan) and water available *ad libitum*. Female mice (N = 37, 8–12 weeks old) were used after the surgical procedures as described below. The temperature (22 ± 2 °C) and humidity (35 ± 5%) in the room were kept constant and a 12:12-h light:dark cycle was maintained with lights turned on at 07:00. All procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23; revised 2011) and approved by the Animal Research Committee of the Obihiro University of Agriculture and Veterinary Medicine.

The induction of sexual receptivity in female mice was according to the previous report (Raskin et al., 2009) with some modification. All females were bilaterally ovariectomized and implanted with a silicone tube (outer diameter, 3 mm; inner diameter, 2 mm; length 20 mm; AS ONE, Osaka, Japan) filled with 30 μL of β-estradiol 3-benzoate (1.7 μg/μL; Sigma-Aldrich, MO, USA) dissolved in olive oil (Wako, Osaka, Japan) at least 1 week before the start of the test. Each female was injected subcutaneously with 100 μL progesterone (10 μg/μL; Wako) 4 h before each test. To confirm sexual receptivity, each female was housed with a sexually experienced male for 10 min, 1 h before each mating test. When the female displayed the lordosis behavior, defined as a posture with ventral arching of the spine without escaping or looking back, we recognized the female mouse was sexually receptive. Immediately after confirmation of sexual receptivity, the female mouse was transferred into her home cage to minimize the influence on the following test.

Mating test

Mating tests were performed between 17:00 and 19:00. Each male was housed individually in a plastic cage (182 × 260 × 128 mm; $l \times w \times h$) at least one day before the start of mating test. Males were separated into three groups: normal males, fed males, and fasted males. Normal males were housed with a pellet diet and water available *ad libitum.* Fed males were deprived of food pellets for 24 h, followed by free-feeding for 8 h prior to testing. Fasted males were deprived of food pellets with water available *ad libitum* for 24 h prior to testing (Fig. 1). A sexually receptive female was transferred into the test cage of each subject, and they were housed together for 30 min. All behaviors were videotaped, and an experienced observer, blinded to treatment, quantified the following parameters during the 30-min test period: the fraction of males mounting a female with or without intromission, the latency before mounting a female without or with intromission, the frequency and duration of males mounting a female without or with intromission, and the fraction of males ejaculating. Mounting without or with intromission was defined as mounting a female without or with pelvic thrusts, respectively. The fraction of males displaying mounts or ejaculation was defined as the number of males showing each behavior at least once for the test period, divided by the total number of males tested. The latency to mount without or with intromission was defined as the amount of time elapsed between placement of the female in the test cage and the first time the male mounted her without or with intromission, respectively. The frequency of mounting without or with intromission was defined as the number of times the male mounted the female without or with intromission during the test period, respectively. The duration of mounting without or with intromission was defined as the total time spent mounting without or with intromission, respectively. If the male never showed mounts during the test period, a latency of 1800 s was recorded. Once used for the test, females were housed individually for at least 3 days before subsequent testing.

Cannula implantation

All procedures were performed according to our previous report (Muroi and Ishii, 2015) with some modifications. Male mice underwent implantation of a stainless steel cannula (Eicom, Kyoto, Japan) using a stereotaxic apparatus (Narishige, Tokyo, Japan) under pentobarbital sodium salt (40 mg/kg body weight, i.p.; Nacalai tesque, Kyoto, Japan) anesthesia. The implanted apparatus consisted of an outer cannula (outer diameter, 0.5 mm; inner diameter, 0.4 mm) and an inner cannula (thickness, 0.35 mm). The length of each cannula was 8 mm for implantation into the cerebral ventricles or 9 mm for the DRN. The tip of the cannula was implanted into the lateral ventricle at bregma-relative coordinates: posterior, 0.7 mm; lateral, 1.1 mm; and depth, 2.1 mm, or into the