Physiology & Behavior 164 (2016) 107–112

Contents lists available at ScienceDirect

Physiology & Behavior

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

The ventromedial hypothalamus oxytocin induces locomotor behavior regulated by estrogen

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HIGHLIGHTS

- The oxytocin system in the VMH is involved in the induction of locomotor behavior.
- Oxytocin is involved in the increase in locomotor activity of proestrus female rats.
- Estrogen facilitates locomotion through oxytocin receptor up-regulation in the VMH.

ARTICLE INFO

Article history:
Received 2 March 2016
Received in revised form 19 May 2016
Accepted 20 May 2016
Available online 27 May 2016

Keywords:
Ventromedial hypothalamus
Locomotor activity
Oxytocin
Oxytocin receptor
Estrogen
Estrus cycle

ABSTRACT

Our previous studies demonstrated that excitation of neurons in the rat ventromedial hypothalamus (VMH) induced locomotor activity. An oxytocin receptor (Oxtr) exists in the VMH and plays a role in regulating sexual behavior. However, the role of Oxtr in the VMH in locomotor activity is not clear. In this study we examined the roles of oxytocin in the VMH in running behavior, and also investigated the involvement of estrogen in this behavioral change. Microinjection of oxytocin into the VMH induced a dose-dependent increase in the running behavior in male rats. The oxytocin-induced running activity was inhibited by simultaneous injection of Oxtr-antagonist, $(d(CH_2)_5^1$, $Try(Me)^2$, $Orn^8)$-oxytocin. Oxytocin injection also induced running behavior in ovariectomized (OVX) female rats. Pretreatment of the OVX rats with estrogen augmented the oxytocin-induced running activity twofold, and increased the Oxtr mRNA in the VMH threefold. During the estrus cycle locomotor activity spontaneously increased in the dark period of proestrus. The Oxtr mRNA was up-regulated in the proestrus afternoon. Blockade of oxytocin neurotransmission by its antagonist before the onset of the dark period of proestrus decreased the following nocturnal locomotor activity. These findings demonstrate that Oxtr in the VMH is involved in the induction of running behavior and that estrogen facilitates this effect by means of Oxtr up-regulation, suggesting the involvement of oxytocin in the locomotor activity of proestrus female rats.

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1. Introduction

Oxytocin, a neuroendocrine hormone, is critical for the promotion of parturition and milk ejection. In addition, recent studies have revealed that oxytocin acts as a neurotransmitter in the central nervous system, involved in many physiological functions. Central oxytocin plays important roles in maternal behavior, reproductive behavior, food intake and anxiety [6,19,36,38]. Oxytocin is synthesized in the paraventricular nucleus (PVN), the supraoptic nucleus (SON) of the hypothalamus, the bed nucleus of the stria terminalis, and the medial preoptic area (MPOA) in rodents [14,39], and modulates the neuronal activation of many brain regions via binding to the oxytocin receptor (Oxtr) [1,10].

Oxtr exists within various brain regions including the PVN, SON, septum, hippocampus, and olfactory bulb [11,21,42]. Oxtr also exists in the ventromedial nucleus of the hypothalamus (VMH) [4,5,48] and is associated with the motor circuit producing the lordosis behavior [12,13]. However, the involvement of Oxtr in other functions of the VMH is not clear.

The VMH plays important roles in the regulation of behavioral, endocrine, and autonomic functions [7,16], and is also known to be involved in producing both instinctive (i.e., feeding and sexual) and emotional (i.e., flight, defense and aggressive) behavior. Running is a commonly shared feature among these behaviors [28]. Thus activation of the subpopulation of VMH neurons via specific receptors could isolate the running behavior from those behaviors ascribed to the VMH [31]. In our previous experiments, running behavior was induced by stimulation of glutamate receptors or by blockade of GABA-A receptors in the VMH of the rat without eliciting feeding, flight, defense or aggressive

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http://dx.doi.org/10.1016/j.physbeh.2016.05.047
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behavior [28–30]. So far, little is known about the involvement of VMH-Oxtr in running activity [34].

Ovarian hormone estrogen is a strong inducer of Oxtr in several tissues including the central nervous system [3,26,50] and this estrogen action is known to control several physiological functions, such as parturition and milk ejection [15,33,37]. The interaction between estrogen and Oxtr in the VMH is shown to participate in regulating female reproductive behavior ([2,5,20,45]. Female locomotor activity is thought to be influenced by estrogen [22,47]. For example, female locomotor activity is at low levels during metestrus and diestrus when circulating estrogen is low, and increases during proestrus as estrogen increases. However, the mechanism underlying the behavioral change has not yet been well-established [8,27]. Therefore it is possible that up-regulation of Oxtr in the VMH could be associated with estrogen action. In this study we investigate the possibility that VMH-Oxtr might participate in inducing locomotor behavior and that estrogen regulates VMH-Oxtr action.

2. Materials and methods

2.1. Animals

Male Wistar rats (Charles River, Yokohama, Japan) and female Wistar–Imamichi rats (Institute for Animal Reproduction, Kasumigaura, Japan) weighing 250–280 g were maintained under controlled conditions of room temperature (22 ± 1 C) and lighting (lights on 0800–2000 h). All rats were 7–8 weeks old at the beginning of the experiment. We used Wistar–Imamichi rats for female experimentation because of their regular four-day estrous cycle [32]. Laboratory chow diet and water were available ad libitum. Food intake and body weight were measured between 10:00 and 11:00 every day. The animals were subjected to hypothalamic implantation of cannulae one week before the experiments, and kept in individual cages until the day of the experiment. Virgin rats were monitored during the estrus cycle by taking vaginal smears each morning. Some female rats were ovariectomized (OVX) under isoflurane anesthesia 1–2 weeks before the brain cannula implantation. All animals were handled for at least one week before the experiments. The entire experiment was reviewed and approved by the Animal Committee for Animal Care at Fukui University.

2.2. Implantation of brain cannula and drug injection

The implantation procedure and VMH drug injection procedure were followed as described previously [29]. Briefly, chronic stainless steel guide cannulae (0.6 mm o.d., 15 mm length) for drug injection were stereotaxically implanted and fixed unilaterally or bilaterally into the VMH of each rat according to the coordinates given in the atlas of Paxinos and Watson [35] under pentobarbital anesthesia (45 mg/kg ip). The implantation coordinates were 6.2 mm anterior to the interaural, 0.7 mm lateral to the midline and 9.4 mm below the dura. A sterile stainless steel stylet was inserted into the guide cannula to prevent the lumen from clotting.

Oxytocin (1.0 or 1.7 μg/μl) (BACHEM, CA, USA) or Oxtr antagonist (10 μg/μl) ($d(CH_2)_5^1$, $Try(Me)^2$, Orn^8)-oxytocin (BACHEM) dissolved in saline were injected into the VMH by means of the brain cannula. Oxytocin (1.0 μg/μl) was also administered in combination with Oxtr antagonist (1.0 or 8.0 μg/μl). The injection was performed unilaterally during 10:00–14:00 in male and OVX female rats or bilaterally before the lights-off in the estrus cycling rats. Previously we reported that unilateral injection of excitatory drug into the VMH induced running behavior [28]. Therefore we employed unilateral injection in this experiment to investigate the effect of oxytocin in inducing running behavior. The drug injection into the VMH was done once in each rat. The injection volume was 600 nl. Drug injection procedures were performed in free moving animals. Following the injection, the cannula was kept in place for an additional 1 min and then replaced with the stylet.

OVX rats were given a subcutaneous injection of 17β-estradiol (12.5 μg, Nacalai Tesque, Kyoto, Japan) dissolved in 0.2 ml of corn oil (OIL) at 11:00–11:20 24 h and 48 h before brain injection.

2.3. Analysis of running activity

We used the running wheel apparatus (FDM-700A, MELQUEST, Toyama, Japan) to analyze running activity, as described previously [31]. It is consisted with the running wheel (diameter = 32 cm, width = 15 cm) and the standard cage in which the rat was able to rest, drink and take food. The rat is able to move freely between the running wheel and the ordinary cage via a hole (25 × 25 cm). The running activity was quantitatively analyzed by counting the revolutions of a running wheel. Each revolution was detected by a photoencoder with a resolution of 1/10 revolution, and the counts were recorded in a personal computer every 30 min.

2.4. Oxtr mRNA quantification in the VMH

OVX rats were given a subcutaneous injection of 17β-estradiol (12.5 μg) dissolved in 0.2 ml of OIL at 11:00–11:20 and were euthanized at 11:00–12:00 the next day. Virgin rats were euthanized at 10:00–12:00 of diestrus (D), proestrus (P AM) and estrus (E), and 16:00–17:00 of proestrus (P PM). Complementary DNA (cDNA) synthesis was performed as described previously [24]. Briefly, VMH punch was performed by stainless tubing (0.8 mm i.d., 2 mm thick). Brain tissue was homogenized in TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and total RNA samples were prepared according to the manufacturer's instructions. RNA samples were treated with DNase I (Invitrogen) and were reverse transcribed using 100 U of ReverTra Ace (TOYOBO Co., Osaka, Japan) and 10 pmol of a 6-mer random primer. Real-time PCR was performed using SYBR Green master mix and an ABI PRISM 7000 sequence detector (Applied Biosystems, Foster City, CA, USA). Previously described reaction protocols and Oxtr-primers (5′-CGATTGCTGGGCGGTCTT-3′ and 5′-CCGCCGCTGCCGTCTTGA-3′ [23]) were used for each PCR assay. Oxtr levels were standardized by dividing Oxtr mRNA values by the value for β-actin in the same sample.

2.5. Histology

At the end of the experiment, VMH injected animals were anesthetized with isoflurane and terminated by decapitation. The brain was excised and fixed by 10% formalin. Coronal sections (25 μm thick) were cut on a freezing microtome and stained with thionin, and the injection site was identified by microscopic histological examination.

2.6. Statistics

The data in each experimental group were first statistically analyzed with ANOVA, and differences between mean values were further analyzed by Tukey–Kramer test. Student *t*-test was also used when it was appropriate. Differences were considered to be significant at $P < 0.05$.

3. Results

The effects of oxytocin injected into the unilateral VMH of male rats on running activity are shown in Fig. 1. Wheel running began immediately after oxytocin injection and lasted for about 30 min (Fig. 1a). The oxytocin-induced running activity increased in a dose-dependent manner and simultaneous injection of an Oxtr antagonist, ($d(CH_2)_5^1$, $Try(Me)^2$, Orn^8)-oxytocin, with oxytocin significantly attenuated running to the control level (Fig. 1b). The injection of the vehicle (left column) induced little running activity.

Oxytocin also increased running in OVX female rats (Fig. 2a). Oxytocin injection into the VMH of estradiol-pretreated OVX rats elicited significantly more running activity than in the OIL-pretreated

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