Available online at www.sciencedirect.com
SCIENCE DIRECT
Brain Research 1043 (2005) 124–131
BRAIN RESEARCH
www.elsevier.com/locate/brainres

Research report

Acute estrogen potentiates excitatory responses of neurons in rat hypothalamic ventromedial nucleus

L.-M. Kow*, A. Easton, D.W. Pfaff

Lab. of Neurobiology and Behavior, The Rockefeller University, 1230 York Avenue, Box 336, New York, NY 10021-6399, USA

Accepted 23 February 2005
Available online 1 April 2005

Abstract

In a previous behavioral study, brief application of a membrane-limited estrogen to neurons in rat hypothalamic ventromedial nucleus (VMN) facilitated lordosis behavior-inducing genomic actions of estrogen. Here, electrophysiological recordings from single neurons were employed to characterize these membrane-initiated actions. From rat hypothalamic slices, electrical activity was recorded from neurons in the ventrolateral VMN, the cell group crucial for estrogen induction of lordosis. In addition to the resting activity, neuronal responses to histamine (HA) and *N*-methyl-D-aspartate (NMDA) were also recorded before, during, and after a brief (10–15 min) application of estradiol (E, 10 nM). These two transmitters were chosen because their actions are mediated by different mechanisms: HA through G protein-coupled receptors and NMDA by ligand-activated ion channels. Vehicle applications did not affect either resting activity or neuronal responses. In contrast, acute E exposure modulated neuronal responses to transmitters, with no significant effect on the resting activity. It potentiated excitatory responses to HAs (20 out of 48 cells tested) and to NMDA (10 out of 19 cells), but attenuated inhibitory responses to HA (3 out of 6 units). Both of these hormonal actions would increase VMN neuronal excitation. In separate experiments, neuronal excitation was found to be suppressed by anesthetics, which would block E's induction of lordosis when administered at the time of estrogen application. These data are consistent with the notion that increasing electrical excitation of VMN neurons can be a mechanism by which acute E exposure facilitates the lordosis-inducing genomic actions of estrogens.

Theme: Neural basis of behavior
Topic: Hormone control of reproductive behavior

Keywords: Estrogen; Neurons; Ventromedial nucleus

1. Introduction

Classically described in endocrine tissues [34], estrogen receptor functions have been elucidated both through transcriptional assays and through the use of null mutations [9]. The molecular properties of these receptors in the cell nucleus are known to depend on certain other nuclear proteins known as co-activators and co-repressors [24]. Using the molecular endocrinology of genomic actions of estrogens helped in unraveling the circuitry for lordosis behavior [26], the first vertebrate behavior to be analyzed successfully in this way.

Recently, however, inspired in part by rapid hormone actions on neurons, membrane-limited effects of estrogens have received more intense study [12]. From the cell membrane, estrogenic hormones can activate a variety of intracellular signaling pathways [19,32]. Since, in turn, many of these pathways are capable of modulating transcriptional actions, we earlier hypothesized that estrogens' rapid membrane actions can synergize with their genomic actions. This hypothesis was verified by in vitro transient transfection studies using neuronal [36] and breast cancer cell lines (Devidze et al., submitted for publication) and by in vivo behavioral study [16]. All of these results were

* Corresponding author. Fax: +1 212 327 8664.
E-mail address: kowl@mail.rockefeller.edu (L.-M. Kow).

0006-8993/$ - see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.brainres.2005.02.068

applied to the theory of specific hormone-facilitated mating behavior dependent on cellular mechanisms in the hypothalamic ventromedial nucleus (VMN).

More generally, it is understood that underlying all emotional behaviors are the mechanisms for generalized CNS arousal [29]. How might generalized arousal impact hypothalamic neurons managing a behavior which reflects a specific form of arousal, such as sexual arousal? To answer this, we measured the effects of two transmitters, histamine (HA) and *N*-methyl-D-aspartate (NMDA), which have been shown to influence all aspects of arousal [27], including sexual arousal [4–6], on the electrical activity of neurons in VMN which govern lordosis, the primary female sex behavior in rodents. Then, we applied estradiol directly into the tissue bath to see if a sex hormone could amplify the effects of these transmitters on VMN neurons. These two transmitters were chosen also because they use different signaling mechanisms: HA through G-protein-coupled receptors [1,10,11,20] and NMDA via ligand-activated ion channels [3,13,31,33]. Finally, since the requirement of neuronal activity for estrogen's lordosis-inducing action was implied by the finding that anesthetization at the time of estrogen application blocked lordosis induction [30], we determined the effect of anesthetics in the tissue bath on VMN neuronal responsiveness to the transmitters.

A portion of the results has been reported in 2002 Society for Neuroscience Annual Meeting.

2. Methods

2.1. Animals

Adult (8–11 weeks) ovariectomized Sprague–Dawley rats were used. They were kept in an air-conditioned room under a reversed light/dark cycle with lights off from 10:00 through 22:00, during which neurons are active and all recordings were carried out. Regular rat chow and water were provided ad libitum. After arrival, the rats were allowed at least 1 week to adapt to the housing. They were then ovariectomized and used for experiments 5–10 days after surgery. All procedures in handling and treating the animals were approved by The Rockefeller University's *Animal Care and Use Committee* in accordance with the Animal Welfare Act and the DHHS "Guide for the Care and Use of Laboratory Animals".

All the methods employed in the present study have been described in our previous reports [14,17,18].

2.2. Preparation of hypothalamic slices

Rats were deeply anesthetized with urethane (16 mg/kg BW) and decapitated. The brain was quickly removed, cooled in ice-cold artificial cerebrospinal fluid (ACSF), and then dissected to obtain hypothalamic block. The block was sliced at 400 μm in ice-cold ACSF with a Vibratome (model 1000 Plus, The Vibratome). Hypothalamic slices containing the ventromedial nucleus were split at the midline and saved in ACSF at room temperature for 1 h before being used for recording. The ACSF consisted of (in mM): NaCl 124, KCl 5, KH_2PO_4 1.2, $MgSO_4$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 26, and glucose 10. It was always saturated with a mixture of 95% O_2 and 5% CO_2 with resulting pH at 7.35 ± 0.05.

2.3. Electrophysiological recording

Hypothalamic slices, one at a time, were placed on the net in the recording chamber, which was continuously perfused with warmed (33–34 °C) ACSF. The slice was submerged in ACSF to facilitate the access of O_2 and test agents. Using glass electrodes filled with ACSF (5–8 MΩ), neuronal electrical discharges were recorded from the ventrolateral portion of VMN, where the density of neurons containing estrogen receptors is high [28]. Action potentials were monitored with a dual beam oscilloscope and were converted to square waves through a window discriminator. The information was then fed into a histogram generator and the firing rate histogram generated was displayed on an event recorder. Neuronal activity was also recorded on tape and in a computer for off-line analyses using pClamp software (Axon Instruments) and MiniAnalysis (Synaptosoft). Only healthy units, as judged from the action potential waveform, were studied. Recordings for such units could routinely last for the entire testing procedure or 1–2 h. To avoid the possibility of sensitization or desensitization by E, each slice was used to study only one unit.

2.4. Experimental procedures

After the firing rate of a neuron stayed stable for 5 min or longer, a dose of histamine (HA) or NMDA was administered. A response was measured by subtracting the resting average firing rate from the peak rate after HA application. The resting rate was averaged from neuronal activity during the 2 min just before HA application. Two or more minutes after the neuron recovered from a response, 17β-estradiol (E, 10 nM) was applied for 10–15 min. At 10 min of application, HA administration was repeated while E was continuously applied. E application was terminated only after the neuron recovered from the HA response. Ten or more minutes later, HA administration was again repeated to observe the recovery from an E effect (potentiation or attenuation). When necessary, HA administration was repeated until the recovery was obvious. To avoid any effects of desensitization or sensitization, HA administration was repeated at an interval of 10 min or longer, a sufficiently long interval from our experience with these hypothalamic neurons. Depending on how soon it recovered from E treatment, a neuron was treated, either with HA alone or paired with NMDA (see below), a total of 3–5 times.

In some experiments, both HA and NMDA were administered. The order of the administration was alternated