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Analyses of rapid estrogen actions on rat ventromedial hypothalamic neurons

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ABSTRACT

Rapid estrogen actions are widely diverse across many cell types. We conducted a series of electrophysiological studies on single rat hypothalamic neurons and found that estradiol (E2) could rapidly and independently potentiate neuronal excitation/depolarizations induced by histamine (HA) and N-Methyl-D-Aspartate (NMDA). Now, the present whole-cell patch study was designed to determine whether E2 potentiates HA and NMDA depolarizations – mediated by distinctly different types of receptors – by the same or by different mechanisms. For this, the actions of HA, NMDA, as well as E2, were investigated first using various ion channel blockers and then by analyzing and comparing their channel activating characteristics. Results indicate that: first, both HA and NMDA depolarize neurons by inhibiting K^+ currents. Second, E2 potentiates both HA and NMDA depolarizations by enhancing the inhibition of K^+ currents, an inhibition caused by the two transmitters. Third, E2 employs the very same mechanism, the enhancement of K^+ current inhibition, thus to rapidly potentiate HA and NMDA depolarizations. These data are of behavioral importance, since the rapid E2 potentiation of depolarization synergizes with nuclear genomic actions of E2 to facilitate lordosis behavior, the primary female-typical reproductive behavior.

1. Introduction

It is now well established that estrogens have at least two kinds of actions, classically defined genomic actions and membrane-initiated rapid actions. The latter are less understood. Rapid estrogen actions can affect a wide variety of cell and tissue types [1,2], ranging from blood cells [3,4], bone cells [5–10], muscle cells [11–15], cancer cells [16–19], cell lines [20–22], to neurons either mature [23,24], embryonic [25] or primary cultured [26]. In addition, estrogens are known to modulate a variety of different signaling systems [27–30].

To examine some of estrogens' actions on neurons of importance for reproductive behavior, we investigated how they affect the responses of female rat hypothalamic ventromedial nucleus (VMN) neurons to histamine (HA) and N-Methyl-D-Aspartate (NMDA). These two neurotransmitter agents were chosen because they induce depolarizing responses via distinctly different receptor types. HA induces depolarization/excitation via a G_q-coupled receptor, and hyperpolarization/inhibition through $G_{i/o}$-coupled receptor [31,32], while NMDA causes depolarization/excitation via a ligand-gated ion channel that does not need the activation

Abbreviations: 4-AP, 4-Aminopyridine; ACSF, artificial cerebrospinal fluid; AP, action potential; DPN, [2,3-Bis-(4-hydroxy-phenyl)-propionitrile; E2, 17β-estradiol; ER, estrogen receptor; ERα, estrogen receptor subtype α; ERβ, estrogen receptor subtype β; H1R, H1R and H1R, histamine receptor subtypes H1, H2 and H3, respectively; HA, histamine; NMDA, N-Methyl-D-aspartate; mER, membrane estrogen receptor; NMDAR, NMDA receptor; PPT, (4-Propyl-[1H]-pyrazole-1,3,5-triyl) trisphenol; TEA, tetraethylammonium; TTX, tetrodotoxin; Vh, holding potential; Vm, membrane potential; VMN, hypothalamic ventromedial nucleus.

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of a G-protein [33]. Our initial study identified 3 different effects of rapid estradiol (E2) action in VMN neurons: potentiation of HA excitatory effects, attenuation of HA inhibition, and potentiation of NMDA effects [34]. These findings raised the possibility that E2 employed three different potential mechanisms to exert these rapid actions on VMN neurons.

In the follow-up neuropharmacological analyses using specific HA agonists and antagonists [35], we confirmed that HA depolarization (which would lead to excitation) is mediated by HA receptor subtype H1 (H1R) while HA hyperpolarization (which would lead to inhibition) was mediated by H2R or H3R. As expected from our initial study, the "pure" depolarization evoked with a specific H1R agonist was potentiated rapidly by E2. But surprisingly, pure hyperpolarization evoked with a H2R or a H3R agonist was neither attenuated nor potentiated by E2. This allows us to eliminate one of the three potential rapid E2 action mechanisms in VMN neurons.

In the present study, whole-cell patch recording was used to examine and compare the rapid E2 actions on the depolarizations induced by HA and by NMDA, and found that there are several close similarities.

2. Methods

2.1. Materials

HA and NMDA were dissolved first in saline as stock solution (50 or 100 mM) and then dissolved in bathing solutions to concentrations to be used. Ion channel blockers and their concentrations used were described at their mention. E2 and testosterone (used as a control for structurally similar E2) were dissolved initially in 100% ethanol and then diluted in distilled water to 1 mM as stock solution and finally in artificial cerebrospinal fluid (ACSF) to the concentrations to be used before experiments. The vehicle for the steroids has been tested in our previous studies and has no effect by itself on neuronal activity. Chemicals and their concentration in bathing solutions were described at their mentioning. All chemicals and test agents for electrophysiology were purchased from Sigma.

2.2. Animals

Intact 12- to 30-day old Sprague-Dawley female rats were used. The age range was chosen to guarantee successful patch clamp recordings from hypothalamic ventromedial nucleus (VMN) neurons. Also, females at these ages have little or no circulating estrogens (absence of estrogen inducible progesterone receptors in the hypothalamus) at least in the brain, but were capable of responding to estrogen, as monitored behaviorally and histochemically [36]. 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 Department of Health and Human Services' "Guide for the Care and Use of Laboratory Animals".

2.3. Brain slices preparation

Hypothalamic slices containing VMN were prepared as described previously [34,37]. Briefly, rats were deeply anesthetized by urethane (160 mg/100 g B.W.) and decapitated to remove the brain. After removing the pia mater from the ventral surface, the hypothalamus was blocked out and placed on the cutting stage of a Vibratome (model 1000 Plus, The Vibratome Company, St Louis, MO). Thin (200–300 μm) coronal slices containing the VMN were cut and collected based on anatomical landmarks. For the blocking and slicing, we used ice-cold sucrose ACSF, in which all the NaCl was replaced by sucrose so as to prevent damage of neurons from over excitation. Slices were then stored in ACSF at room temperature for at least 1 h before recording. The ACSF is composed of (mM): NaCl, 126; KCl, 5; NaH_2PO_4, 1.25; $CaCl_2$, 2; $MgCl_2$, 2; $NaHCO_3$, 26; dextrose, 10; and oxygenated with 95% O_2/5% CO_2.

2.4. Whole-cell patch recording

One slice at a time was placed on the bottom of the recording chamber fixed to the stage of an upright microscope (Olympus BX50WI) and superfused with ACSF at 1.5–2 ml/min at room temperature. The slice was examined with infrared differential interference contrast (IR-DIC) optics. All the neurons recorded in the present study were from the ventrolateral portion of the VMN, which is rich in neurons with estrogen receptor and is critically important for estrogen induction of lordosis (Pfaff, 1980). To accomplish this, ventrolateral VMN was located under low magnification (40×) before switching to higher magnification (400×). At the end of an experiment the location of the recorded neuron was re-affirmed with low magnification. Under high magnification, cells were visually selected and patched conventionally with electrodes (2–5 MΩ) pulled from Borosilicate glass pipettes (G8515OT-4, Warner Instruments, Hamden, CT) with a Narishige PP-830 puller and filled with an internal solution, which was composed of (mM): K-Gluconate, 140; EGTA, 5; $MgCl_2$, 2; $NaHCO_3$, 0.6; HEPES, 10; Mg-ATP, 2; Na_2-ATP, 2; $CaCl_2$,1; Na-GTP, 0.3; and sucrose, 8.3. Whole-cell current or membrane potential was amplified with Axoclamp 200A initially and Multiclamp 700B later in this study and recorded and analyzed with Clampex and Clampfit, respectively (Axon Instruments). The extracellular solution was ACSF, unless otherwise indicated. Once a patch was obtained, membrane test was performed to check following criteria: access resistance ⩽15 MΩ, leak current ⩽30 pA and membrane potential (Vm) to be equal or more negative than −45 mV. Once a criterion was violated the recording was terminated. All experiments were performed under current clamp.

2.5. General experimental procedure

The Vm was then observed for 5 min or longer and only neurons with stable Vm were studied. To ensure that the patched cell is a neuron, it was injected briefly with a depolarizing current to evoke action potentials (APs). In experiments using tetrodotoxin (TTX), action potentials were maintained with continuous current injection, if necessary, while TTX was administered to assure that the Na^+ channel blocker was effective. In those without TTX, the holding membrane voltage was set to −65 mV or more hyperpolarized if necessary, in attempts to avoid the spontaneous occurrence of AP.

A test agent, HA, NMDA or E2 and related agents was then applied repetitively either through the bath or by picospritzer. With bath application, the recording chamber perfusion was switched from ACSF to a modified ACSF containing a test agent. With this method, a response is usually small and slow (see Fig. 1).

The picospritzer (Spritzer-8, Cornerstone) was used only for administering HA or NMDA. The responses induced by spritz are usually much greater and much rapid than those by bath application (compare Fig. 1 with Fig. 2, note the difference in time scales). HA or NMDA (1–50 mM, pH 7.4–6.05, in ejecting pipette) was ejected with a picospritzer (2.5×10^5 Pa for 0.1–20 s) near the patched neuron. To control for pH, 7 neurons were picospritzed with ACSFs with pH ranging from 7.05–6.05, equivalent to HA concentrations 5–50 mM. None of the 7 neurons was affected, neither depolarized nor hyperpolarized, by any of the ACSFs. In a previous study [35], we found the use of picospritzer was safe: mechanical

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