17-β-Estradiol Modulation of Area Postrema Potassium Currents

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Li, Zhicheng and Meredith Hay. 17-β-Estradiol modulation of area postrema potassium currents. J Neurophysiol 84: 1385–1391, 2000. The purpose of this study was to determine the effects of 17-β-estradiol on area postrema neuronal activity in vivo and on area postrema potassium currents (IK) in vitro. In anesthetized rats, intravenous injection of 17-β-estradiol (10 ng/kg bw) -inhibited area postrema neuronal activity in 8/8 neurons tested. The averaged firing rate decreased from 2.9 ± 1.1 to 1.1 ± 0.3 Hz. The inhibitory effects of 17-β-estradiol on area postrema neuronal activity were rapid in onset (within 1 min) and long-lasting (>8 min). To study the cellular mechanisms involved in this response, the effects of 17-β-estradiol were examined in dissociated area postrema neurons. In these cells, 17-β-estradiol (0.5 nM) increased the averaged peak IK 27 ± 8%. The time course for the potentiation was observed within ~0.5–1 min after the application of 17-β-estradiol. Full recovery from the potentiation usually occurred within ~3–4 min after the washout of 17-β-estradiol. The biologically inactive 17α-estradiol had no effect on area postrema IK and the 17-β-estradiol antagonist,ICI 182,780 blocked the effects of 17-β-estradiol on area postrema IK. Finally, big conductance calcium-activated potassium current (MaxiK\(^+\)) was identified in area postrema neurons (n = 12/12). Blockade of MaxiK\(^+\) with 100 nM iberiotoxin blocked the effects of 17-β-estradiol on IK. These results suggested 17-β-estradiol might modulate area postrema neuronal activity by increasing MaxiK\(^+\) current.

INTRODUCTION

Estrogen, especially 17-β-estradiol, has long been used as a replacement hormone in postmenopausal women to achieve a wide range of health benefits, including among others, cardiovascular protection against hypertension and stroke (Stampfer et al. 1991). However, little is known about the estrogen’s effects on the CNS or nuclei within the CNS involved in cardiovascular regulation.

Circumventricular organs are unique central structures that allow for the communication of information between circulating hormones and peptides and the CNS. The area postrema is a circumventricular organ in the hindbrain which is known to be important in many physiological functions including the central regulation of cardiovascular function. The area postrema is known to send dense projections to the nearby dorsomedial and dorsal lateral nucleus tractus solitarius (Morest 1967; Shapiro and Miselis 1985; van der Kooy and Koda 1983), lateral parabrachial nucleus, and the dorsal motor nucleus of the vagus (Shapiro and Miselis 1985), all of which are known to be important for the autonomic neuronal activity control. The central projections from the area postrema to these nuclei have been suggested to be important for the cardiovascular regulatory effects of area postrema activation.

Estrogen receptors have been identified in the rat area postrema (Laflamme et al. 1998) and have been suggested to be involved in the regulation of area postrema neuronal activity. The purpose of present study was to 1) determine the role of estrogen on area postrema neuronal activity, and 2) to begin to evaluate some of the cellular mechanisms underlying 17-β-estradiol’s modulation of area postrema neurons.

METHODS

In vivo electrophysiology

Intact female Sprague-Dawley rats (150–300 g) were anesthetized with urethane (0.6 g/kg). Animals were randomized as to their stage in the estrous cycle. The responses of area postrema neurons to an acute dose of 17-β-estradiol were similar in all animals tested, regardless of the stage in the estrous cycle. Femoral arterial and venous catheters (PE-50 Intramedic) were inserted to monitor blood pressure and to deliver drugs, respectively. Animals were placed in a stereotaxic head holder (Kopf). The area postrema was exposed by removing the atlantooccipital membrane between the occipital bone and first vertebrae. Animals were allowed to breathe spontaneously. An automatic heating pad was used to maintain rat body temperature at 37 ± 1.0°C.

Area postrema single-unit activities were recorded using glass-recording electrodes (~5–10 MΩ, filled with 3.0 M sodium chloride). Extracellular single-unit recordings were obtained to a maximal depth of 400 μm around the center of area postrema and to 200 μm around the edge of area postrema. Electrical signals were amplified on a Grass P5 amplifier, discriminated via WPI window discriminator, and then digitized using MacLab (Chart software). A minimum 2-min spontaneous single-unit activity was recorded before the administration of 17-β-estradiol (10 ng/kg). This single dose of 17-β-estradiol was selected to mimic the maximal total circulating estradiol a female rat might reach during proestrus (Smith et al. 1975). The effect of 17-β-estradiol administration on the spontaneous activity of an area postrema single-unit activity was then evaluated. An excitatory or inhibitory response is defined by a ±25% increase or decrease in neuronal firing frequency, respectively. Histological locations of the recorded neurons were obtained by ejecting 2.5% Chicago blue through a injection pipette attached to the recording electrode.

At the end of experiment, the animal was euthanized and perfused intracardially with phosphate-buffered saline followed by 4% formalin. The hindbrain was stored in 4% Formalin plus 30% sucrose solution overnight. The locations of recording electrode tracts in the AP were verified histologically.

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Ten-day-old Sprague-Dawley rats of mixed gender were used in all the described studies. The hindbrain and cerebellum were rapidly removed and placed in 4°C physiological buffer containing (mM) 124 NaCl, 5 KCl, 2.0 CaCl₂, 1.0 MgCl₂, 26.0 NaHCO₃, 10.0 glucose, pH 7.35. A 500-μM-thick, horizontal medullary slice, which included the area postrema, was obtained using a vibratome. Under a dissecting microscope, the area postrema was easily visualized and cut away from the surrounding tissue. The tissue was incubated at 37°C for 30 min in an Earl's balanced salt solution containing 5 mg/ml papain (Sigma), 1.5 mg/ml bovine serum albumin. The tissue was triturated in a papain-free solution with serially smaller pipettes until most of the tissue was dissociated. Dissociated cells were rinsed in minimum essential media (MEM) and plated on poly(lysine)-coated coverslips. Cells were maintained in MEM with 8 ng/ml nerve growth factor (Gibco).

**Patch-clamp recordings**

All experiments were performed on area postrema neurons following 2–3 days in culture before the growth of extended neurites. Patch-clamp electrodes were constructed from No. 8161-type capillary glass (WPI Glass) pulled on a Flaming/Brown micropipette puller and polished on a Narishige microforge. Final electrode resistance was between 1–3 MΩ. Standard whole-cell patch-clamp techniques used were similar to those described by other investigators (Hamill et al. 1981). Patch pipettes filled with the appropriate solutions were attached to the head-stage amplifier, which was mounted to a hydraulic micromanipulator (Narishige). The reference electrode consisted of an Ag–AgCl plug immersed in a 150 mM KCl agar bridge, which was placed in the bath. Recordings were made using an Axopatch 1-D patch clamp amplifier and filtered at 3 kHz using a four-pole Bessel filter. Currents were digitized on-line at 10 kHz using Axodata software (Axon Instruments) and stored on a Macintosh computer for analysis. Current-voltage relationships were corrected for linear leakage current measured from hyperpolarizing command pulses from −90 to −120 mV. Junction potentials were electronically compensated and balanced to zero with the pipette immersed in the bath solution. Data are reported as mean ± standard error. Results were analyzed using t-test or ANOVA test.

**Pharmacological application**

Experiments were performed on cells which had been plated onto 9-mm-square coverslips. The coverslips were placed in a recording chamber filled with 260 μM bath solution. The flow of fluid (2 ml/min) through the changer was controlled by a multiport solenoid valve system that governed the gravity-feed flow onto the cell.

**Solutions**

Potassium currents (IK). (All solution components were obtained from Sigma.) Ion substitution and pharmacological agents were used to isolate the K⁺ current. The bath solution consisted of (in mM) 137.0 N-methyl-D-glucamine, 5.4 KCl, 0.2 CaCl₂, 1.0 MgCl₂, 10.0 glucose, 10.0 HEPES, pH 7.35–7.40. The pipette solution consisted of (in mM) 145.0 KAsp, 1.0 MgCl₂, 10.0 HEPES, 0.2 CaCl₂, 10.0 EGTA, and pH 7.20. The final calcium concentration in the pipette solution is 1.0 μM (Fabiato 1988).

**RESULTS**

17-β-estradiol inhibits area postrema neuronal activity

Figure 1A is a photomicrograph illustrating the recorded area postrema neurons stained with 2.5% Chicago blue. Figure 1B is an example of area postrema single-unit recording. In the present study, a total of eight area postrema neurons were recorded and tested with 17-β-estradiol injection. The frequency of area postrema spontaneous neuronal activity ranged from a minimum 0.2–9.9 Hz. The average rate of discharge of

**FIG. 1. Labeling of recording site within the area postrema. A: region from which area postrema recordings were obtained. Arrows indicate area postrema neurons which were labeled with microinjection of 2.5% Chicago blue. B: single-unit action potential recording from an area postrema neuron.**

Intravenous injection of 17-β-estradiol inhibited area postrema neuronal activity in all eight neurons (Fig. 2B), an example of which was shown in Fig. 2A. As summarized in Fig. 2B, 17-β-estradiol decreased the average firing rate by 62%, from 2.9 ± 1.1 to 1.1 ± 0.4 Hz (P < 0.05). These effects on area postrema neuronal activity were rapid in onset (within 1 min, Fig. 2A) and long-lasting (>8 min).

17-β-estradiol facilitates area postrema IK

The modulation of voltage-gated IKs has been shown to be important in the regulation of neuronal excitability. 17-β-estradiol has been shown to modulate certain types of IKs (Joels and Karst 1995). To determine the specific effects of 17-β-estradiol on area postrema neuronal activity, we studied the voltage and pharmacological properties of IKs in area postrema neurons in the presence of 17-β-estradiol using whole-cell patch-clamp methodology.

Figure 3A is a 3-day-cultured area postrema neuron. Figure 3B illustrates a typical total IK isolated from an area postrema neuron evoked by 10-mV step depolarizations from −80 mV holding potential to +30 mV. Figure 3C demonstrates the voltage-current relationship of this area postrema IK.

Figure 4 illustrates the effect of 17-β-estradiol on evoked area postrema IK. Application of 17-β-estradiol (50 nM) potentiated IK. Five minutes after washout of 17-β-estradiol and replacement of the bath solution, peak IK recovered toward the control level. In all the neurons tested (n = 13/13), application of 17-β-estradiol (50 nM) potentiated IK at every voltage level (Fig. 4D).

The effects of 17-β-estradiol on area postrema IK were dose related. An example is shown in Fig. 5A. For the generation of accumulative concentration-response curve, 17-β-estradiol was applied to an area postrema neuron consecutively from low to high concentration with 10-fold increases. In other experiments, some cells were lost during increasing 17-β-estradiol concentrations, thus not all concentrations were tested in a single cell. The responses to 17-β-estradiol were normalized within each cell to the control peak IK level and averaged across cells. Although variability analysis suggests that the effects at different concentration levels were not statistically different, there was an obvious trend toward an increase of effects with increasing concentrations.

Selectivity of 17-β-estradiol facilitation of area postrema IK

17-α-estradiol has the same molecular formula as 17-β-estradiol but it does not have any known physiological function. ICI 182,780 is an estrogen antagonist that does not have
a partial agonist effect on estrogen receptors. The unique pharmacological properties of ICI 182,780 were used to determine whether the effects of 17-β-estradiol on area postrema IK were specific for 17-β-estradiol activation of an estrogen receptor. As shown in Fig. 6A, 17-α-estradiol had no effect on area postrema IK-evoked depolarization from −80 mV holding potential to 20 mV. The averaged data are shown in Fig. 6B (n = 4). To test whether 17-β-estradiol’s effects on area postrema IK were due to the binding of the agent to area postrema estrogen receptors, 1 μM ICI 182,780, together with 17-β-estradiol (50 nM), was applied to the bath solution. As demonstrated in Fig. 6C, in the presence of ICI 182,780, 17-β-estradiol did not have any effects on area postrema IK. The averaged results were shown in Fig. 6D (n = 4).

17-β-estradiol modulation of area postrema big conductance calcium-activated IK (MaxiK+)

Using a specific MaxiK+ channel blocker, iberiotoxin, we were able to isolate MaxiK+ in area postrema neurons. As shown in Fig. 7A, 800-ms, 10-mV step depolarizations from −80 mV holding potential evoked a series of area postrema IK. In Fig. 7B, 100 nM iberiotoxin was applied to the bath solution to block MaxiK+. The before- and after-iberiotoxin area postrema potassium peak IKs were plotted against their corresponding depolarization voltages in Fig. 7D. Figure 7E illustrates digitally subtracted MaxiK+ current. The MaxiK+ activated near −30 mV and increased its peak amplitude linearly with more positive depolarization voltages. MaxiK+ was found in all area postrema neurons tested (n = 12/12). The MaxiK+ constitutes from 39 to 68% of total area postrema IK, with an average contribution of 52 ± 5%. These results suggested that MaxiK+ might play an important role in area postrema neuronal activity.

To test whether 17-β-estradiol can selectively potentiate area postrema MaxiK+, the following protocol was followed: 1) A neuron was first tested with 17-β-estradiol and allowed to recover. 2) The same neuron was then treated with iberiotoxin to block the MaxiK+ current. 3) A second application of 17-β-estradiol together with iberiotoxin was applied to the neuron to determine whether the facilitory effects of 17-β-estradiol could still be observed during blockade of MaxiK+. As shown in Fig. 8A, area postrema IK evoked by an 800-ms depolarization to 20 mV was potentiated by 17-β-estradiol by 30%. Application of 100 nM iberiotoxin revealed a MaxiK+ of 0.63 nA, which was 60% of the total IK. The second application of 17-β-estradiol...
diol in the presence of iberiotoxin did not increase the remaining IK. Averaged data are illustrated in Fig. 8B. These results suggest that 17-β-estradiol inhibits area postrema neuronal activity by increasing MaxiK$^+$ current.

**DISCUSSION**

The results from this study are the first to demonstrate that a sex hormone can modulate area postrema neuronal activity and area postrema IK channel function. Furthermore, these results suggest that 17-β-estradiol may inhibit area postrema activity via nongenomic mechanisms.

17-β-estradiol’s modulation of area postrema neuronal activity is most likely to involve excitation or inhibition of its IKs, a major determinant of neuronal excitability. Previous studies have shown that area postrema neurons possess at least two types of IKs 1) a rapid-activating and rapid-inactivating (IA current) and 2) a delayed rectifier current which is slowly activating and noninactivating (Hay and Lindsley 1995). In the present study, we have shown area postrema neurons also express an iberiotoxin-sensitive Ca$^{2+}$-activated potassium channel, the MaxiK$^+$ channel. The MaxiK$^+$ is a category of big conductance (15–250 pS) potassium channels that is sensitive to both changes in intracellular calcium concentration and membrane potential. In neurons, MaxiK$^+$ contributes to the afterhyperpolarization that modulates repetitive firing and overall neuronal excitability. The large presence of MaxiK$^+$ in area postrema neurons might underlie the exhibited low level of spontaneous activity observed in these cells.

In reports from other laboratories, the effects of 17-β-estradiol on IKs have been varied and appear to depend on the cell type that has been tested. For example, 17-β-estradiol can stimulate IA-type transient outward currents (Rusko et al. 1995) in rabbit aortic endothelial cells but inhibit the IA-type transient currents in rat myometrial cells (Erulkar et al. 1994). However, it does not affect the IA-type current in rat hippocampal neurons (Joel and Karst 1995) nor delays an inward rectifier current expressed on *Xenopus* oocytes (Waldegger et al. 1996). In the present study, area postrema neurons show an increase in their total IK to 17-β-estradiol application. Our results are in agreement with a number of studies which show 17-β-estradiol increases IK and results in hyperpolarization.
(Kelly et al. 1980; Nabekura et al. 1986). However, in area postrema neurons, 17β-estradiol does not change IA or the delayed rectifier current. Thus, MaxiK+ is the principal component responsible for the area postrema total IK increase observed with 17β-estradiol. Our results are similar to observations in cardiac myocytes and coronary artery smooth muscle cells where 17β-estradiol activates MaxiK+ (Node et al. 1997a,b; Wellman et al. 1996). Since MaxiK+ activation requires an elevation of [Ca2+], one explanation for this enhanced MaxiK+ is the intracellular calcium surge resulting from activation of calcium channel by 17β-estradiol (Joels and Karst 1995). However, it has yet to be tested whether 17β-estradiol can affect area postrema voltage-gated Ca2+ channels.

In the present study, both the in vivo and in vitro neuronal response to 17β-estradiol were rapid. The increase of area postrema IK was observed 45 s after 17β-estradiol and maximal responses appeared within ~1–2 min. Similar rapid responses to 17β-estradiol have been observed in other studies on medial amygdala neurons (Nabekura et al. 1986). In the present study, ICI 182,780, an estrogen receptor antagonist without partial estrogen bioactivity, can totally block the effects of 17β-estradiol on area postrema IK. These results suggest that estrogen receptors participate in mediating the action of 17β-estradiol in area postrema neurons. However, the exact signal transduction pathway of 17β-estradiol’s effects is yet to be determined.

Since the genomic effects of estrogen require a complex cascade of events including the hormone-receptor binding, targeted gene expression, and protein synthesis, it may take hours for the hormone signal to be translated into membrane excitability changes. The time course of the response in the present study suggests that genomic effects are unlikely and nongenomic effects are the reasonable alternative mechanism underlying 17β-estradiol’s action in these studies. In contrast to genomic mechanism, the nongenomic effects are rapid in onset and do not require nuclear estrogen receptors or gene expression. However, it does need membrane receptors and/or other cellular second-messenger systems to change neuronal activity (Node et al. 1997a). MaxiK+ channels comprise two subunits: the α subunit, which forms the pore of the channels (Adelman et al. 1992; Butler et al. 1993; Tseng-Crank et al. 1994), and the β subunit, which forms the regulatory site sensitive to intracellular Ca2+ (McManus et al. 1995; Meera et al. 1996). Recently, 17β-estradiol has been shown to directly bind to the β subunit of MaxiK+ expressed on Xenopus oocytes and can acutely activate this channel (Valverde et al. 1999). These studies in the Xenopus oocyte may suggest a total new mechanism by which 17β-estradiol may modulate MaxiK+ channel activity.

The area postrema, one of seven circumventricular organs, has the highest estrogen receptor density among cardiovascular-related brain nuclei (Laflamme et al.1998). Functionally, the area postrema is known to be involved in regulation of body fluid balance, feeding behaviors, emesis, and cardiovascular regulation (Shapiro and Miselis 1985). It sends dense projections to the nearby dorsomedial and dorsal lateral nucleus tractus solitarius, lateral parabrachial nucleus, and the dorsal motor nucleus of the vagus (Morest 1967; Shapiro and Miselis 1985; van der Kooy and Koda 1983), all of which are known to be important for the autonomic control of blood pressure. Several lines of evidence have suggested that circulating 17β-estradiol may modulate autonomic and cardiovascular function in particular via CNS modulation of sympathetic outflow (Akaishi et al. 1996a,b; Chu and Beilin 1997; Colucci et al. 1982; Condon et al. 1989; Crofton and Share 1997; He et al. 1998). Because of the area postrema’s dense population of estrogen receptors, its known involvement in the modulation of sympathetic activity, and its inhibition by circulating estradiol, it is reasonable to suggest that the area postrema may be one central site whereby circulating estradiol may act to modulate sympathetic outflow and potentially cardiovascular function. Future studies will be designed to determine the role of the area postrema in estradiol modulation of cardiovascular regulatory function.

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