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17β-ESTRADIOL INHIBITS OUTWARD POTASSIUM CURRENTS RECORDED IN RAT PARABRACHIAL NUCLEUS CELLS *IN VITRO*

M. FATEHI,[a,b] S. B. KOMBIAN[c] AND T. M. SALEH[a,b]*

[a]*Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, P.E.I., Canada C1A 4P3*

[b]*Prince Edward Island Health Research Institute, University of Prince Edward Island, Charlottetown, P.E.I., Canada*

[c]*Department of Applied Therapeutics, Faculty of Pharmacy, Kuwait University, Safat, Kuwait*

Abstract—Evidence is increasingly accumulating in support of a role for the steroid hormone 17β-estradiol to modify neuronal functions in the mammalian CNS, especially in autonomic centers. In addition to its well known slowly developing and long lasting actions (genomic), estrogen can also rapidly modulate cell signaling events by affecting membrane excitability (non-genomic). Little, however, is known regarding the mechanism(s) by which 17β-estradiol produces its rapid effects on neuronal membrane excitability. As potassium channels play a crucial role in cell excitability, we hypothesized that 17β-estradiol caused excitability by modulating potassium flux through the neuronal cell membrane. We tested this hypothesis by examining the effects of 17β-estradiol on outward potassium currents recorded in cells from the parabrachial nucleus of rats, *in vitro*. Bath application of 17β-estradiol (10–100 μM) reversibly reduced voltage-activated outward potassium currents in a concentration-dependent manner. This effect was mimicked by BSA-17β-estradiol but not mimicked by 17α-estradiol and was significantly reduced by ICI 182,780, a selective estrogen receptor antagonist. The inhibitory effect of 17β-estradiol was dependent on extracellular potassium concentration, with more profound effects observed at lower concentrations. The 17β-estradiol-induced inhibition of the outward current was blocked by pretreatment with the potassium channel blockers tetraethylammonium and 4-aminopyridine. The time constants of deactivation of tail currents were decreased by 17β-estradiol over a range of test potentials (−140 to −80 mV). Finally, the inhibitory effect of 17β-estradiol on the outward potassium currents was blocked following pre-incubation of slices in lavendustin A, a tyrosine kinase inhibitor. Taken together, these results suggest that 17β-estradiol acts rapidly at an extracellular membrane receptor to reduce tetraethylammonium- and 4-aminopyridine-sensitive outward potassium currents by accelerating the closure of potassium channels. This may be the ionic basis of 17β-estradiol-induced enhancement of neuronal excitability. © 2005 Published by Elsevier Ltd on behalf of IBRO.

*Correspondence to: T. M. Saleh, Prince Edward Island Health Research Institute, Department of Biomedical Sciences, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, P.E.I., Canada C1A 4P3. Tel: +1-902-566-0819; fax: +1-902-566-0832.
E-mail address: tsaleh@upei.ca (T. M. Saleh).

Abbreviations: aCSF, artificial cerebrospinal fluid; DMSO, dimethyl sulfoxide; Erev, reversal potential; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; I–V, current-voltage; PBN, parabrachial nucleus; TEA, tetraethylammonium; TTX, tetrodotoxin; V_h, holding potential; 4-AP, 4-aminopyridine.

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The parabrachial nucleus (PBN) is an important autonomic regulatory nucleus that serves to integrate and relay visceral information projecting to the thalamus and cortex (Chamberlin and Saper, 1992; Huang et al., 2003; Kang et al., 2004; Saleh and Cechetto, 1994). Immunocytochemical evidence exists for the presence of both estrogen receptors (ERα and ERβ) on cell bodies, axons and terminals of autonomic regulatory nuclei throughout the neuraxis including the PBN (Turcotte and Blaustein, 1993). In addition to the receptors, endogenous estrogens are also present in these nuclei (Saleh et al., 2003). The presence of estrogen and its receptors on cells in these autonomic nuclei suggest that estrogen may exert rapid regulatory and modulatory effects on integrated responses and information to and from these nuclei. It is believed that 17β-estradiol influences neuronal communication and subsequently cardiovascular function through different mechanisms, ranging from trans-synaptic modulation of neurotransmitter synthesis and release, to development and remodeling of synaptic circuitry, and via the response of the target postsynaptic cell. Despite the presence of estrogen receptors in these cardiovascular regulatory nuclei, their role in the function of these nuclei is not well known. The modulatory role of 17β-estradiol in the PBN has been the subject of recent investigations in our laboratory (Saleh and Connell, 2003a,b; Saleh et al., 2005). Bilateral microinjection of 17β-estradiol into the PBN was shown to rapidly (within 30 min) decrease sympathetic nerve activity and increase the sensitivity of the cardiac baroreceptor reflex (Saleh et al., 2000, 2003; Saleh and Connell, 2003a).

At the cellular level, the effects of estrogens on neuronal excitability and synaptic transmission are also not well understood. Microinjection of estrogen into the PBN of male rats while recording extracellular neuronal activity in the thalamus *in vivo*, rapidly enhanced GABAergic neurotransmission within the PBN and attenuated visceral afferent activity to the thalamus (Saleh and Saleh, 2001). Long-term, genomic effects of estradiol have also been observed with responses occurring hours or days following pretreatment. For example, it has also been reported that estrogen enhanced sensorimotor performance following 2 week implantation of estradiol containing pellets into the neostriatum (Becker et al., 1987). Also, one week pretreatment of neostriatal neurons with estrogen, caused an enhancement of the firing rate in response to dopamine in these cells (Arnauld et al., 1981). Much of what is known about the rapid (within 5 min of application), cellular effects

of estrogen within the CNS comes from studies in hypothalamic and hippocampal neurons. It has been shown that 17β-estradiol decreased the spontaneous firing of medial preoptic area neurons (Kelly et al., 1977), enhanced the firing rate of pituitary cells (Dufy et al., 1979) and potentiated excitatory postsynaptic potentials in hippocampus (Wong and Moss, 1992).

Thus, although the effects of 17β-estradiol on neuronal function in several regions of the brain are widespread, the underlying mechanism(s) of action remain uncertain. Generally, the most reported effects of estrogen on the electrophysiological properties of CNS neurons are those of increased membrane excitability. To begin to understand the ionic basis of this excitability, we hypothesized that estrogens may modulate potassium conductances. The present study therefore examined the effects of 17β-estradiol, the endogenously bioactive estrogen, on voltage-activated potassium currents recorded from parabrachial neurons contained in pontine slice preparations *in vitro*.

EXPERIMENTAL PROCEDURES

Slice preparation

All efforts were made to minimize the number of animals used in this study. The handling and maintenance of animals met the guidelines of the Canadian Council on Animal Care and were approved by the University of Prince Edward Island Animal Care Committee (protocol # 04–032). Male Sprague–Dawley rats (Charles River, Montreal, PQ, Canada) weighing 150–200 g were deeply anesthetized with isoflurane (Abbott Laboratories, Saint-Laurent, PQ, Canada) vapor in a closed environment and then decapitated. After quick removal, the brain was immersed in ice-cold (2–3 °C) artificial cerebrospinal fluid (aCSF) with the following composition (in mM): 126 NaCl, 2.5 KCl, 11 D-glucose, 18 $NaHCO_3$, 1.2 NaH_2PO_4, 1.3 $MgCl_2$, 2.5 $CaCl_2$ (pH 7.4), which was continuously bubbled with 95% O_2–5% CO_2. Coronal slices (300 or 400 μm thick) containing the PBN were prepared from a tissue block of the brain maintained in ice cold carbogenated aCSF, using a vibratome (Model 1000 plus, Ted Pella, Inc., Redding, CA, USA). Slices were incubated in aCSF at room temperature (22–25 °C) for at least 45 min prior to recording. A single slice was then transferred to a 500 μl recording chamber and submerged in continuously flowing extracellular solution (2–3 ml/min) gassed with 95% O_2–5% CO_2.

In vitro electrophysiological recordings

"Blind" whole-cell patch-clamp recordings (Blanton et al., 1989) were performed on cells from the PBN using an EPC-10 amplifier (Heka Electronics Inc., Mahone Bay, NS, Canada) controlled by the Pulse (data acquisition and analysis) software program (Heka Electronics Inc.). All experiments were carried out at temperatures of 30±1 °C. A tight giga ohm seal on each cell was obtained using micropipettes (6–8 MΩ) pulled from thin-walled (outer diameter, 1.5 mm) borosilicate glass capillaries (World Precision Instruments) by a vertical puller (Model PIP5; Heka Electronics Inc., Germany). The composition of the intracellular (pipette-filling) solution was (in mM): 130 K-gluconate, 6 NaCl, 10 HEPES, 2.5 Na-ATP, 0.1 Na-guanosine 5-triphosphate (pH adjusted to 7.2 with KOH). The extracellular solution had the same composition as that used for the dissection. Sodium and calcium conductances were respectively blocked by adding tetrodotoxin (TTX) (10 nM) and $CdCl_2$ (0.1 mM) to the external solution. The fast electrode capacitance was first compensated. After achieving whole-cell configuration, capacitance transients were cancelled by using an automatic compensation function of the EPC-10 (about 70–80%) and were monitored periodically. Access resistance (series resistance) was also carefully monitored throughout the experiment and only those cells that showed less than 10% change in access resistance (ranged 20–25 MΩ) over the period of experiments were included in the analysis of the data. Following adequate access to the cell, the amplifier was switched to voltage clamp mode. Data acquisition and analysis were performed using Pulse and Pulsefit software (Heka Electronics Inc.), respectively. Current–voltage relationships (I–V curves) were generated as described below. Currents were filtered at a frequency of 1 kHz. Membrane currents were subjected to leak subtraction to remove the passive component of the membrane conductance. This was performed on-line using the p/4 method (Bezanilla and Armstrong, 1977), and applied by activating this protocol in the Pulse software. Tail currents were fitted by a single exponential function to measure their time constant of decay using a Pulsefit program (Tristani-Firouzi and Sanguinetti, 1998; Wigmore and Lacey, 2000). All cells were voltage clamped at a holding potential (V_h) of −65 mV near their resting potential. Voltage-dependent outward potassium currents were elicited by applying depolarizing voltage pulses. Acquired cells were pulsed up to +60 mV (50 ms duration) in voltage steps of 10 mV from the V_h unless otherwise stated. A family of outward currents was recorded under control conditions and then at 5 min intervals following drug exposure. The shift in the I–V relationship was measured as the voltage difference between the curve under control conditions and 5 min after exposure to 17β-estradiol (0.1–100 μM) and taken as the estrogen effect. All drugs were applied to the cells by bath perfusion of the slices with aCSF containing the final concentration of the drug.

Statistics

Data are expressed as mean percentage change from control values±standard error of the mean (S.E.M.). Each individual cell served as its own internal control. For comparison of various groups, means were analyzed by one-way analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison test. Results expressed as percentages of control were considered to be nonparametric data and analyzed by employing the Mann-Whitney *U* test. Statistical significance was determined at $P<0.05$. Graphing was performed using the SigmaPlot®, GraphPad® and CorelDraw software®.

Drugs

Isoflo (isoflurane) was purchased from Abbott Laboratories. Cadmium chloride and ICI 182,780 were obtained from Fisher Scientific (Fair Lawn, NJ, USA) and Tocris (Ellisville, MO, USA), respectively. 17α-Estradiol, 17β-estradiol, BSA-17β-estradiol, lavendustin A, TTX, tetraethylammonium (TEA), 4-aminopyridine (4-AP), and all the salts in the aCSF were purchased from Sigma (St. Louis, MO, USA). When 4-AP and TEA were included in the extracellular solution, the pH was adjusted to 7.4 by using HCl. Appropriate stock solutions were made and diluted with aCSF just before application.

RESULTS

Bath application of low concentrations of 17β-estradiol (<50 μM) at the V_h of −65 mV did not induce any measurable change in the resting (holding) current. This suggests that 17β-estradiol does not alter a resting or leak conductance to excite PBN neurons. However, 17β-estradiol at a high concentration (50 μM) caused a slight, but significant cell membrane depolarization (8±3 mV; 12.8±2.2% of control, $P<0.05$; $n=5$). We therefore studied depolarization-induced potassium currents.