Estrogen suppresses epileptiform activity by enhancing Kv4.2-mediated transient outward potassium currents in primary hippocampal neurons

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Received February 12, 2015; Accepted July 8, 2015

DOI: 10.3892/ijmm.2015.2287

Abstract. Catamenial epilepsy is a common phenomenon in female epileptic patients that is, in part, influenced by the 17-β-estradiol level during the menstrual cycle, which modulates the strength of the epileptic seizures. However, the underlying mechanism(s) for catamenial epilepsy remains unknown. In the present study, the effect of 17-β-estradiol on modulating epileptiform activities was investigated in cultured hippocampal neurons by focusing on the transient outward potassium current. Using the patch clamp technique, 17-β-estradiol was demonstrated to have a dose-dependent U-shape effect on epileptiform bursting activities in cultured hippocampal neurons; only the low dose (~0.1 ng/ml) of 17-β-estradiol had a suppressive effect on the epileptiform activities. The blockade effect of the low dose 17-β-estradiol could be suppressed by phrixotoxin2 (PaTx2), a selective channel blocker for voltage-gated potassium channel type 4.2 (Kv4.2), which mediates the transient outward potassium current. Furthermore, the 17-β-estradiol bell-shape-like dose-dependently enhanced the transient outward potassium current, which was inhibited by the estrogen receptor antagonist ICI 182,780. In conclusion, these results indicate that reduced activation of the transient outward potassium current by a high (or none) 17-β-estradiol level may enhance the epileptiform bursting activities in neurons, which may be one of the triggering causes for catamenial epilepsy, and therefore, maintaining a certain low 17-β-estradiol level may aid in the control of catamenial epilepsy.

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Key words: potassium channel, Kv4.2, estrogen receptor, epilepsy

Introduction

Epilepsy is one of the most common diseases of the central nervous system. In certain female patients, seizures may cluster in association with the menstrual cycle, and this is known as catamenial epilepsy (1). The association between seizures and fluctuations of the sex hormone levels during the ovarian cycles in certain women with epilepsy was suggested as early as the 19th century (2). Since then, clinicians have investigated this observation further and identified that it may be attributed to the neuroactive properties of steroid hormones and their cyclic variation in serum levels. However, the association between estrogens and epileptic seizures remains unclear (3).

Voltage-gated potassium (Kv) channels are important physiological regulators of membrane potentials, action potential shape, firing adaptation and neuronal excitability in excitable tissues (4). The voltage-gated potassium (Kv) currents are divided into two sections: The transient current and the sustained current (5,6). Previous studies have demonstrated that the transient outward potassium current, also known as the A-type potassium current (I_A), has an important role in controlling the membrane excitability and it contributes to remodel neuronal excitation under pathological conditions (7,8). In the Kv channel family, Kv4 is the major subtype mediating the A-type current (I_A). I_A is encoded by homomultimeric or heteromultimeric complexes of the Kv channel subunits within identical subfamilies (9,10). In the hippocampus, dendrites of CA1 pyramidal neurons contain a high density of transient A-type potassium channels, and this dampening effect reduces the ability of dendrites to initiate action potentials, decreases the amplitude of back-propagating action potentials, and reduces the magnitude of EPSPs (11). Taken together, these reveal that the A-type potassium current has an inhibitory modulation effect on neuronal activities in the hippocampus, which suggest that inhibition of Kv channels would increase the cell excitability. Indeed, in pilocarpine seizure rat model, by using electrophysiological recording, the function of A-type potassium channels were found to be decreased, which most likely accounts for the increased dendritic excitability and the epileptic seizure generation (12).

In one of our previous studies, we reported that phenol red, a weak estrogen receptor agonist, as well as 17-β-estradiol, by

activation of estrogen receptors, exhibited a dose-dependent U-shape-like effect on the spontaneous epileptiform bursting activities in cultured hippocampal neurons (13); however, the underlying mechanism remains unknown. Hoffman *et al* (14) also reported that modulation of voltage-gated potassium channels by estrogen reduced seizure and induced the spread and degree of neuronal loss in the kainate acid (KA) epilepsy model, which was modulated by progesterone (15). Previous studies have shown that estrogen receptor activation affects the function of various potassium channels in the brain. 17-β-estradiol has been reported to inhibit the activity of the outward potassium currents in the rat parabrachial nucleus (16) and modulate the small conductance calcium-activated SK3 potassium channels in the hypothalamus of the female guinea pig (17). In GT1-7 cells, estrogen has been reported to enhance the Kv4.2 mRNA expression level and transient outward current (18). Thus, activation of estrogen receptors is likely to affect the neuronal excitability and in turn reduces the occurrence of epilepsy, and potassium channels may be involved in this process. However, the mechanism of how estrogen receptor activation modulates the transient outward potassium channel and affects the epileptiform activities is not fully understood.

Therefore, we hypothesized that estrogen modulation of epileptiform activity may occur, at least in part, via affecting the transient outward potassium current. In the present study, the effect of estrogen on the excitability and outward potassium currents of cultured hippocampal neurons was investigated. The results showed that 17-β-estradiol has a suppressive effect on the epileptiform bursting activities in cultured hippocampal neurons and an enhancing effect on the transient outward potassium currents with the similar dose-related U-shape manner. Inhibition of the transient outward potassium currents reversed the low dose 17-β-estradiol-evoked inhibition on epileptiform bursting activities, indicating that the transient outward potassium currents mediated the anti-seizure effect of low dose 17-β-estradiol.

Materials and methods

Ethics statement. All the animal experiments were approved by the Local Committees of the Use of the Laboratory Animals, Fudan University (Shanghai, China) and were carried out in accordance with the National Natural Science Foundation of China animal research regulation.

Primary hippocampal neuronal culture. Primary hippocampal neurons were prepared from embryonic day 18 Sprague-Dawley rats, as previously reported (19). Briefly, the pregnant rat was anaesthetized with 10% chloral hydrate (intraperitoneal injection), and the pups were dissected out for tissue preparation. All the animals were subsequently euthanized with an overdose of chloral hydrate. Following the dissection of the hippocampus, the tissue was rinsed in cold Hanks' balanced salt solution and subsequently digested with 0.05% trypsin-ethylenediaminetetraacetic acid for ~15 min at 37˚C, followed by trituration with pipettes in the plating media (Dulbecco's modified Eagle's medium with 10% fetal bovine serum, 10% F12 and 25 μg/ml penicillin/streptomycin). Subsequent to rinsing twice, cells were counted and plated onto glass coverslips (12-mm round; Carolina Biological Supply Co., Burlington, NC, USA) or 35-mm petri dishes with a 20-mm glass bottom well (Shengyou Biotechnology Co., Ltd., Hangzhou, China) precoated with 0.1 mg/ml poly-D-lysine (Sigma Aldrich, St. Louis, MO, USA). Subsequent to culturing for 1 day, half of the media was changed into neuronal culture media (neurobasal media containing 2 mM GlutaMAX^{TM}-I supplement, 2% B27 and 25 μg/ml penicillin/streptomycin) without phenol red. Ara-C (2 μM; Sigma-Aldrich) was added 6-8 days after plating during the culture medium change, and cells were fed twice weekly thereafter. All the cells were grown at 37˚C and in 5% CO_2.

Drugs and treatment. Hippocampal neurons were cultured with phenol red-free neurobasal cell culture medium throughout the whole culture period. Different concentrations of 17-β-estradiol (0, 0.1 and 1 ng/ml) were added into the cell culture medium at 1, 3, 6 and 10 days *in vitro* (DIV), to maintain the neuron development and survival until the analysis. For the drug treatment study, the estrogen receptor antagonist ICI 182,780 (100 nM) (20,21) and selective Kv4.2 and Kv4.3 channel blocker phrixotoxin2 (PaTx2) (100 nM) (22) were added to the culture medium with 17-β-estradiol at the DIV1 for co-culturing.

Patch-clamp recordings data acquisition. Whole-cell recordings of pyramidal shaped neurons were recorded using a conventional patch-clamp technique with MultiClamp 700B amplifier (Axon Instruments, Foster City, CA, USA). Patch pipettes were pulled using a Sutter P-97 (Sutter Instrument, Novato, CA, USA) pipette puller. The bath solution contained 128 mM NaCl, 5 mM KCl, 25 mM HEPES, 1 mM $MgCl_2$, 30 mM glucose and 2 mM $CaCl_2$ (pH adjusted to 7.3 with NaOH). Soft-glass recording pipettes were filled with an internal solution containing 125 mM potassium gluconate, 10 mM KCl, 10 mM HEPES, 5 mM EGTA, 10 mM Tris-phosphocreatine, 4 mM MgATP and 0.5 mM Na_2GTP (pH adjusted to 7.2-7.3 with KOH). The pipette resistance was 3-5MΩ subsequent to filling with internal solution. The cultured pyramidal shaped neurons selected for electrophysiological recording exhibited the same morphological characteristics. All the recordings were performed at room temperature.

In order to record the action potential, the membrane potential was held at -70 mV in the current clamp mode. A large depolarization shift was defined as a membrane potential up shift for >10 mV, lasting for ≥300 msec. A bursting activity was defined by ≥5 consecutive action potentials overlaying on the top of this large depolarization shift. When quantifying the percentage of neurons showing bursting activity, the criterion is ≥2 bursts occurring during 10 min of recording, the same as our previous studies (23,24).

I_A recordings were obtained in the voltage clamp mode at -100 mV. To record the fast transient outward potassium currents, the step command potentials were applied between -70 and +40 mV, with 10-mV steps and a width of 200 msec. In order to trigger the A-type potassium current, a prepulse (-100 mV, 200 msec) was applied to the cells immediately prior to the step commands (18). To isolate I_A, tetraethylamonium (5 mM), tetrodotoxin (1 μM) and $CdCl_2$ (100 μM) were added to the extracellular solution to block the calcium channels,

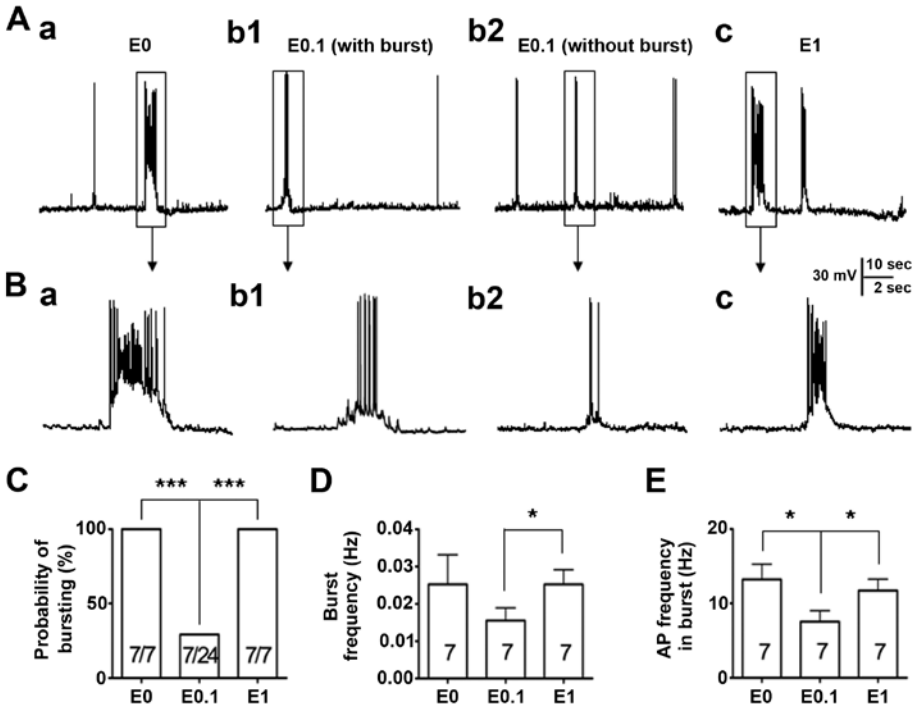

Figure 1. U-shape dose effects of 17-β-estradiol on neuronal burst activities in cultured hippocampal neurons. (A and B) Representative traces showing activities of neurons cultured with (a) 0, (b) 0.1 and (c) 1 ng/ml (A) 17-β-estradiol, and the traces in (B) are the enlarged images from (A) as indicated. (C-E) Bar histograms to show the group analysis of (C) the burst probability, (D) the averaged burst frequency and (E) the averaged inner burst action potential frequency in these three groups. Number of neurons analyzed were as indicated. The comparisons were tested by (C) χ^2 test and (D and E) Student's t-test. *P<0.05 and ***P<0.001.

delayed rectifier potassium channels and voltage-gated sodium channels.

All the electrophysiological data were recorded using a MultiClamp 700B amplifier (Axon Instruments). Data acquisition and analysis were performed with pClamp 10.2 software (Axon Instruments).

Equation for curve fitting of the steady-state activation of I_A currents. In the activation experiments, membrane potential was first held at -100 mV. The total voltage-dependent potassium currents were evoked by a 200 msec depolarization pulse from -70 to +40 mV in 10-mV steps at 10-sec intervals. The I_A current value was the difference between the instant peak current and the steady-state current. Data were analyzed by calculation of the equation $G = I/(V_m-V_{rev})$, for the membrane potassium conductance. V_m represents the membrane potential, whereas V_{rev} is the reversal potential of K^+ (25). Subsequent to normalizing each current amplitude to the maximal current amplitude obtained from the depolarization to +40 mV, the function $G/G_{max} = 1/\{1 + \exp[-(V_m - V_{1/2})/k]\}$ was used to fit the data. From this equation, an activation curve of I_A was obtained and the $V_{1/2}$, the voltage at which the I_A current was half-activated, was calculated.

Data analysis. All the electrophysiological results were analyzed using two-way analysis of variance (ANOVA) for comparisons between multiple groups, and post hoc analysis was performed with Fisher's least significant difference, χ^2 test and the Student's t-test for direct comparison between the two groups. Data were all presented as mean ± standard error of the mean and analyzed by SPSS 13.0 software (SPSS, Inc., Chicago, IL, USA). P<0.05 was considered to indicate a statistically significant difference.

Results

U-shape dose-dependent effect of 17-β-estradiol on epileptiform bursting activities in cultured hippocampal neurons. In a previous study, we reported that phenol red, a weak estrogen receptor agonist, at the concentration of a supplement ingredient in the commercially available culture medium, has a suppressive action on epileptiform bursting activities in cultured neurons by activation of the estrogen receptors (13). Thus, in the present study, all the experiments were performed in phenol red-free medium cultured hippocampal neurons. In the phenol red-free neurobasal culture medium, DIV14 hippocampal neurons were cultured with alcohol (as the vehicle control for 17-β-estradiol) or 17-β-estradiol at doses of 0.1 and 1 ng/ml, and were patch-clamp recorded in the current mode to record their firing properties (Fig. 1). Similar to the previous study (13), all the neurons (7/7, 100%) were showing epileptiform bursting activities while the neurons were cultured in phenol red-free medium with no added 17-β-estradiol (0 ng/ml). However, when the neurons were cultured in low dose 17-β-estradiol (0.1 ng/ml)-added medium, the percentage of neurons showing abnormal epileptiform bursting discharges (7/24, 29%) was significantly reduced (P<0.001) when compared to the vehicle control. By contrast, when the neurons were cultured in the high dose of 17-β-estradiol (1 ng/ml), the percentage of neurons showing epileptiform bursting

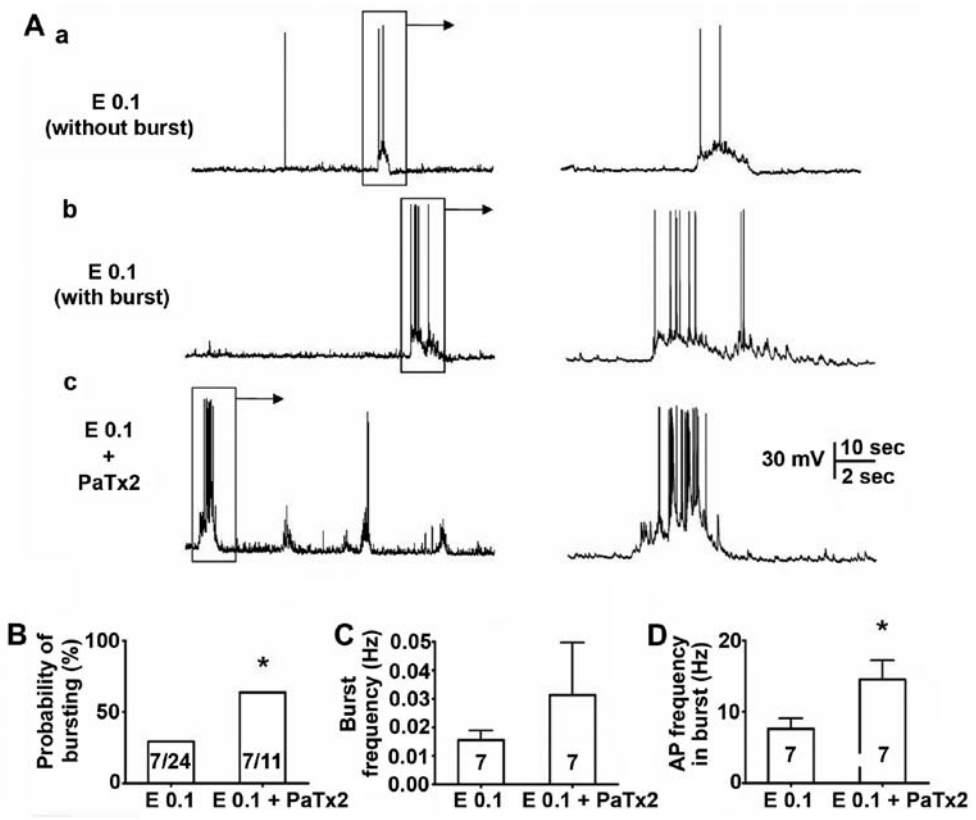

Figure 2. Inhibition of Kv4.2 channel with phrixotoxin2 (PaTx2) on the low dose 17-β-estradiol-induced suppressive effect on epileptiform burst activities. (A) Representative traces of the neuronal activities in the groups of neurons cultured in 0.1 ng/ml 17-β-estradiol (a and b) without or (c) with PaTx2 (100 nM). (B-D) The statistic analysis of (B) the burst probability, (C) the burst frequency and (D) the inner burst action potential frequency in these two groups. Number of neurons studied are as indicated. The comparisons were tested by the Student's t-test. *P<0.05.

discharges (7/7, 100%) was not different to that of the vehicle control group, but significantly more than that of the low dose 17-β-estradiol (0.1 ng/ml) group (P<0.001) (Fig. 1A-C).

In addition, the burst frequency and the action potential frequency were analyzed within the burst among those bursting neurons recorded. The data revealed that when neurons were cultured with 0.1 ng/ml 17-β-estradiol and had burst activities (n=7), the burst frequency was not different to that of the neurons in the vehicle control group (P>0.05) (Fig. 1D); however, the action potential frequency within the burst was significantly lower (P<0.05) than that in the neurons in the vehicle control group (Fig. 1E). The burst frequency and the action potential frequency were further compared within the burst between the bursting neurons from neurons cultured with either low (0.1 ng/ml) or high (1 ng/ml) 17-β-estradiol medium. The results showed that the burst frequency and the action potential frequency within the burst among those bursting neurons were significantly lower (P<0.05) in neurons treated with 0.1 ng/ml 17-β-estradiol compared with those in 1 ng/ml 17-β-estradiol group (Fig. 1D and E).

These results indicate that the effect of 17-β-estradiol on the epileptiform bursting discharges is U-shape like, and 17-β-estradiol, in a certain low dose range, has a suppressing effect, similar to the effect that we previously reported (13).

Effect of PaTx2 on epileptiform bursting activities. Previous studies have shown that 17-β-estradiol has a modulation effect on the transient (mediated by Kv4.2) and sustained outward potassium current (major mediated by Kv2 family) (18), and Kv4.2 is associated with the seizure occurrence (12). Thus, whether the suppressive effect of the low dose (0.1 ng/ml) 17-β-estradiol on spontaneous epileptiform bursting activities in cultured neurons is due to modulation of the transient outward potassium current mediated by Kv4.2 was investigated using the selective Kv4.2/Kv4.3 channel blocker PaTx2. After 14 days co-culturing in low dose 17-β-estradiol (0.1 ng/ml) with PaTx2 (100 nM), neurons at DIV14 were patch-clamp studied of their spontaneous activities (Fig. 2). As expected, PaTx2 significantly blocked the low dose 17-β-estradiol-induced suppressive effect on the epileptiform activities (Fig. 2A). The percentage of neurons showing epileptiform activity in the PaTx2-treated group was 64% (7 in 11), which was significantly higher than those cultured with low dose 17-β-estradiol alone (29%, 7 in 24; P<0.05) (Fig. 2B). In addition, PaTx2 also significantly inhibited low dose 17-β-estradiol-induced reduction of the average action potential frequency within the burst (P<0.05) (Fig. 2D). It should be noted that we did not compare the effect of PaTx2 and high dose 17-β-estradiol on bursting activity in this study, but aim to do so in the future.

This result indicates that the potassium channel Kv4.2 is possibly involved in the low dose of 17-β-estradiol-induced suppressive effect on epileptiform activities.

Dose-dependent effect of 17-β-estradiol on fast transient outward potassium currents in cultured hippocampal neurons. In order to study the mechanism of how 17-β-estradiol

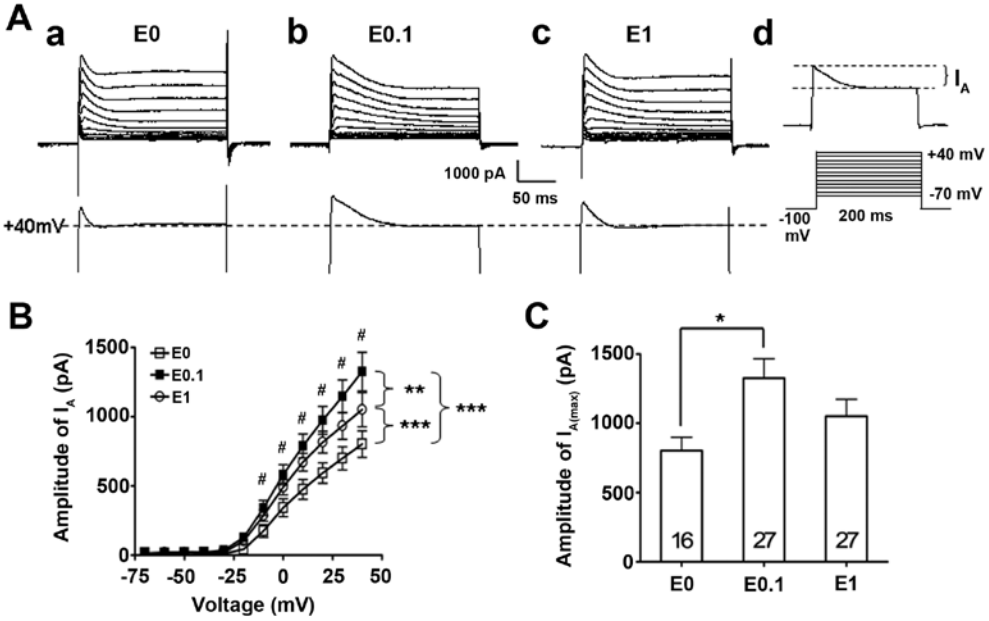

Figure 3. Dose-dependent effects of 17-β-estradiol on potassium I_A currents. (A) Representative original traces of the (a-c) steady-step voltage change-evoked potassium channel currents, and (d) the schematic diagram of I_A measurement and the recording voltage ladder. (a-c) The single traces below are the I_A currents obtained at a membrane voltage of +40 mV. (B) The I-V curves of the I_A change at the voltage step from -70 to +40 mV in the culture groups treated with 17-β-estradiol at 0 (n=16), 0.1 (n=27) and 1 (n=27) ng/ml. **P<0.01 and ***P<0.001, respectively, as tested by two-way analysis of variance, and $^{\#}$P<0.05 represented the comparison between E0 and E0.1 groups by post-hoc analyses. (C) Comparison of the amplitude of maximal amplitude of the I_A ($I_{A(max)}$) tested at +40 mV. *P<0.05 by the Student's t-test.

modulates epileptiform activity of hippocampal neurons through the potassium Kv4.2 channel, the fast transient outward potassium currents (I_A), which are mostly mediated by Kv4.2 channel, were further tested by the patch-clamp technique in different 17-β-estradiol-treated neuron groups. Similar to the effect of 17-β-estradiol on epileptiform bursting activities in cultured hippocampal neurons, a dose-related 'bell' shape-like effect of 17-β-estradiol on fast transient outward potassium currents was identified (Fig. 3). Statistical analysis of the I-V curves of the I_A revealed that low (0.1 ng/ml, n=27) and high dose (1 ng/ml, n=27) 17-β-estradiol significantly enhanced I_A compared to the 17-β-estradiol-free vehicle control group (P<0.001, two-way ANOVA) (Fig. 3A and B). However, further statistical analyses between the low and high dose of the 17-β-estradiol-treated groups data demonstrated that the low dose of 17-β-estradiol (0.1 ng/ml) had the largest enhancement effect on the I_A, while further increasing the 17-β-estradiol concentration to a higher dose (1 ng/ml) caused a reduction, although this remained higher compared to the vehicle control group and did not further enhance on the I_A (P<0.01, two-way ANOVA) (Fig. 3A and B). However, the post-hoc analysis showed that only the low dose of 17-β-estradiol had significant enhancement on the I_A (P<0.05) (Fig. 3B).

The differences of the maximal amplitude of the I_A ($I_{A(max)}$), which was evoked while the current command was at +40 mV, was further studied among those groups. The data showed that the $I_{A(max)}$ was 1,325±139 pA (n=27) in the low dose of 17-β-estradiol (0.1 ng/ml) group, which was significantly higher than that of the $I_{A(max)}$ (802±96 pA, n=16, P<0.05) in the vehicle control group (17-β-estradiol at 0 ng/ml). By contrast, the $I_{A(max)}$ in the high dose of 17-β-estradiol (1 ng/ml) group was 1,050±122 pA (n=27), which was not different to that of the neurons tested in the vehicle control group (P>0.05).

These data indicate that I_A is capable of being modulated by the long-term estrogen receptor stimulation at a low dose of 17-β-estradiol, which enhanced the I_A amplitude.

Effect of ICI 182,780 on the suppressive action of low dose 17-β-estradiol on the I_A currents in cultured hippocampal neurons. As 17-β-estradiol treatment at the low and high concentration had a modulation effect on the potassium I_A, whether the enhancement of the low dose of 17-β-estradiol on the potassium I_A is mediated by the estrogen receptors was further analyzed using ICI 182,780, an estrogen receptor antagonist.

When neurons were co-cultured with 17-β-estradiol at 0.1 or 1 ng/ml and with ICI 182,780 (100 nM), the statistical analysis of the I-V curves showed that antagonism of the estrogen receptors with ICI 182,780 significantly suppressed the low (P<0.05, n=34, two-way ANOVA) (Fig. 4A) and high (P<0.01, n=22, two-way ANOVA) (Fig. 4B) dose 17-β-estradiol-induced potassium I_A enhancement in comparison with the 17-β-estradiol treatment alone groups (n=27 and n=27, respectively) (Fig. 4B and C).

In addition to suppression of the I_A amplitude, the antagonism of the estrogen receptor with ICI 182,780 also significantly left-shifted the steady-state activation curve of the I_A currents, which represents the channel-opening properties of the Kv4.2 potassium channels under the low dose 17-β-estradiol (0.1 ng/ml) culture condition. The I_A current half-activation potential ($V_{1/2}$) underwent a significant depolarization shift increase from -7.48±1.6 mV (n=27) to 0.00±1.8 mV (n=34) (P<0.05) in the neuron groups with 17-β-estradiol (0.1 ng/ml) alone or co-treatment with ICI 182,780, respectively (Fig. 5).

These data indicate that I_A is capable of being modulated by the long-term estrogen receptor stimulation at the low dose of

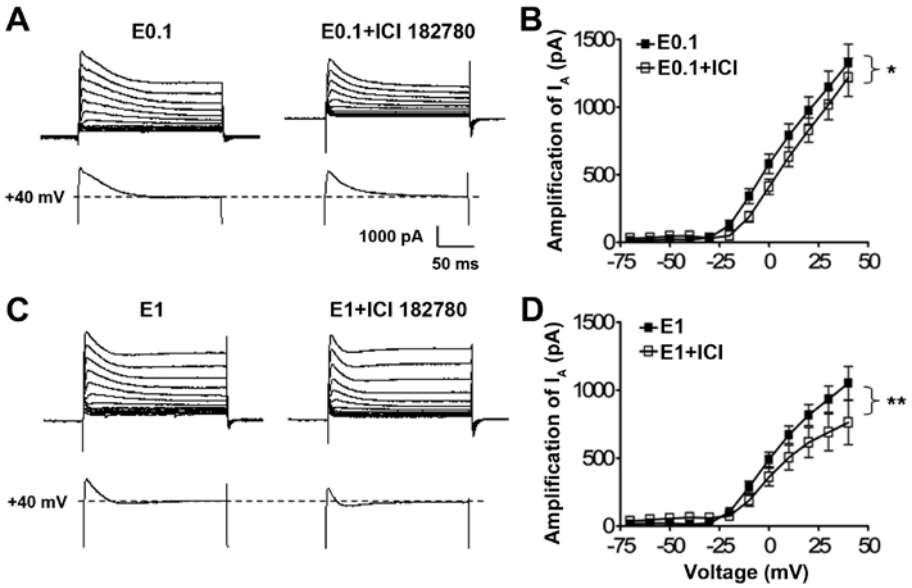

Figure 4. Estrogen receptor antagonist ICI 182,780 inhibits the 17-β-estradiol-induced enhancement of potassium I_A current. (A and C) Representative original traces of potassium currents evoked by the steady-step voltage change from -70 to +40 mV (upper traces), and the single-traces at voltage of +40 mV (bottom traces). (B and D) The I-V curves of the I_A change at the voltage step from -70 to +40 mV in the co-culture groups treated with ICI 182,780 (100 nM) and 17-β-estradiol at either 0.1 (n=34) or 1 (n=22) ng/ml. *P<0.05 and **P<0.01, respectively, as compared with 17-β-estradiol at 0.1 (n=27) and 1 (n=27) ng/ml alone by two-way analysis of variance.

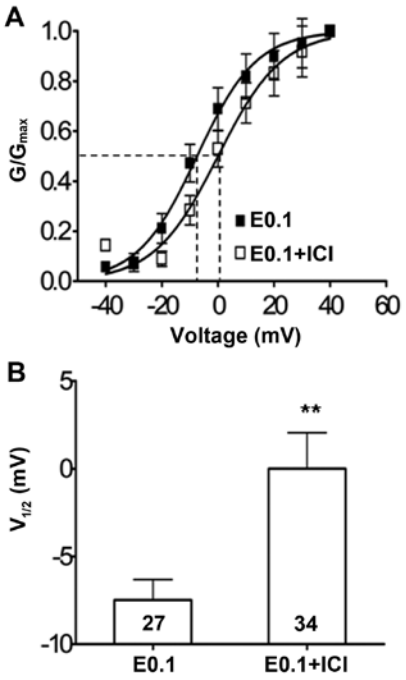

Figure 5. ICI 182,780 alters the I_A steady-state activation curve to a depolarizing level. (A) I_A steady-state activation curves obtained by plotting the normalized conductance as a function of command potential obtained from neuron groups treated with low dose 17-β-estradiol (0.1 ng/ml) (n=27) and with or without estrogen receptor antagonist ICI 182,780 (100 nM) (n=34). Data points were fitted by the Boltzmann function. ICI 182,780 significantly right shifted the steady-state activation curves (G represents conductance). (B) Bar histogram showing ICI 182,780 significantly depolarizing shifted the $V_{1/2}$. **P<0.01.

17-β-estradiol, which enhanced the potassium Kv4.2 channel activities by enhancing I_A amplitude and channel-opening probability.

Discussion

The present experimental results demonstrated that 17-β-estradiol, the biologically active estrogen, had a regulative action on abnormal epileptiform bursting activities in cultured hippocampal neurons in a U-shape dose-dependent manner: Extremely low or high concentrations of 17-β-estradiol enhance, but low concentrations of 17-β-estradiol suppress, the epileptiform bursting activities in the cultured hippocampal neurons. This suppressive effect of 17-β-estradiol on the epileptiform bursting activities is possibly due to, at least in part, the modulation of the potassium Kv4.2 channel activities by activation of estrogen receptors.

In the present study, the dose effect of estrogen receptor stimulation on the epileptiform bursting activities in cultured hippocampal neurons was examined. Estrogen has been reported to have a differential effect on neuronal function. 17-β-estradiol has been reported to increase the excitability of gonadotrophin-releasing hormone neurons (26), medial vestibular nucleus neurons in brain stem (27) and hippocampal neurons (28). By contrast, estrogen receptor activation has also been reported to decrease neuronal excitability by indirectly changing the local neurotransmitter release (29), particularly by changing the interaction with GABAergic neurons (30,31). Our previous study also indicated that on the same hippocampal neuron, a weak estrogen receptor agonist, phenol red, could have a U-shape-like activation-inhibition-activation effect on the epileptiform bursting activities: Low and high concentrations of phenol red all induced the epileptiform bursting activities in the cultured hippocampal neurons, while the middle concentration (~28 μM) of phenol red suppressed this activity (13). Similar to the previous reports, a large proportion of the neurons cultured in the estrogen-free medium had epileptiform bursting activities in the present study, and

the low dose 17-β-estradiol (~0.1 ng/ml) had a suppressive effect, whereas the high dose 17-β-estradiol (1 ng/ml) had a promoting effect on the neuronal excitability change, forming a U-shape-like dose-dependent action on the neuronal excitation change. This effect may explain the differential action of the estrogen receptor stimulation caused increase or decrease of neuronal excitability in various studies (28-31).

Neuron excitability is determined by various factors; one of them is the open properties of the potassium channels. Voltage-gated potassium (Kv) channels are important for maintaining the membrane potentials, action potential shape, firing adaptation and neuronal excitability in neurons (4). The Kv channels-mediated current contains the transient and the sustained current (5,6). Previous studies have demonstrated that the transient outward I_A has an important role in controlling the membrane excitability and that it contributes to remodeling neuronal excitation under pathological conditions (7,8). In the present study, the modulatory effect of low dose 17-β-estradiol on the epileptiform bursting probability was blocked by the selective Kv4.2 and 4.3 potassium channel blocker PaTx2, indicating the involvement of the Kv4.2 and 4.3 potassium channels. The effect of 17-β-estradiol on the voltage-gated fast transient outward potassium current was further examined to improve the understanding of how 17-β-estradiol affects neuronal excitability and the probability of the epileptiform bursting activity occurrence. Using whole cell clamp recordings, the results demonstrated that 17-β-estradiol had a dose-associated modulatory effect on the voltage-gated fast transient outward I_A curve and on the maximal current measured when the membrane potential was transiently increased to +40 mV. The low and high dose of 17-β-estradiol significantly increased the amplitude of the I_A. Notably, the low concentration of 17-β-estradiol at 0.1 ng/ml had the strongest effect on the increase of the amplitude of I_A, which is also significantly more than that of 17-β-estradiol at 1 ng/ml, showing a bell-shape concentration-dependent manner on the facilitation of the voltage-gated fast transient outward I_A. These results are consistent with the finding that a low dose of 17-β-estradiol only had a significant suppressive effect on the epileptiform bursting activities, which showed a U-shape concentration-dependent inhibitory action. As the transient outward I_A has an important role in controlling the membrane excitability and remodeling neuronal excitation under pathological conditions (7,8), the present results indicate that activation of estrogen receptors on modulating neuronal epileptiform bursting activities may, through the mechanism of modulating the transient outward I_A, alter the cell excitability.

Estrogen is one of the main hormones of female mammals (however, it also exists in males), which has a complex and wide physiological and pathophysiological effect, such as promoting cell proliferation (32-34) as well as modulating the neuronal excitability (3,27,28,35-37), and the effect of 17-β-estradiol, an estrogen receptor agonist, observed in the present study is possibly due to activation of the estrogen receptors, as the facilitation effect on the transient outward I_A in cultured hippocampal neurons was inhibited by the estrogen receptor antagonist ICI 182,780 (20,21) (Fig. 4). However, the results from the present study could not distinguish which subtype of the estrogen receptors was mediating this function, as ICI 182,780 is neither an α- nor β-estrogen receptor subtype-selective antagonist. The reason why 17-β-estradiol has an effect on the transient outward I_A, as well as on the neuronal epileptiform bursting activities, in a bell-shape (U-shape) mannor remains unknown. We hypothesize that the estrogen receptor subtype may have a major role in differentially modulating neuronal excitability with regards to the firing properties. The involvement of either the α- or β-subtype estrogen receptors in mediating this 17-β-estradiol action requires further study using selective antagonists for α- and β-estrogen receptor subtypes in the future.

Potassium channels are important for post-excitatory membrane repolarization and sustain different components of hyperpolarizing after-potentials in intrinsically bursting cells. The inhibition of K^+ currents interferes with repolarization and hyperpolarization, and increases hyperexcitability and bursting activity (38). In the Kv channel family, Kv4 is the major subtype mediating the transient outward I_A (9,10). In the hippocampus, dendrites of CA1 pyramidal neurons contain a high density of transient A-type potassium channels, and A-type K^+ channels are crucial modulators of information processing and synaptic plasticity in the dendrites (9,10). A reduction in A-type K^+ channel activity promotes burst firing in a location-dependent manner (39). The changes in the potassium currents alter shape and repetitive frequency of the action potentials. The presence of the rapidly inactivating A-type channels maintains the firing rate at a low frequency and broadens the action potentials when neurons fire repetitively (40). These two changes diminish the firing rate in the neurons. This indicates that the A-type potassium current is a type of inhibitory effect in the normal physiological condition in the hippocampus. In the present study, the neurons with a high probability to have epileptiform bursting, such as neurons in the estrogen-free culture group, also have a high firing frequency of the action potentials in the burst, and the lowest I_A. Increasing the I_A by low dose of 17-β-estradiol significantly reduced the action potential firing frequency along with the inhibited epileptiform bursting probability and the suppressed bursting frequency. This result indicates that there is a negative correlation between the transient outward potassium current and the epileptiform bursting properties, including bursting frequency and bursting action potential frequency. This is consistent with the previous findings that in pilocarpine seizure rats, the increased dendritic excitability and the epileptic seizure generation is possibly due to the decreased A-type potassium channels activities (12).

The results from the present study suggest that estrogen receptor activation is important for modulating the neuronal cell excitability and maintaining the normal neuronal physiological conditions, including the firing properties of the neurons, by modulating the potassium channel activities. As estrogen receptor stimulation could either enhance the neuronal excitability by directly modulating the intrinsic properties (28,36) or, in the other direction, decrease the neuronal excitability by indirectly changing the local neurotransmitter release (29,35) particularly by interaction with GABAergic neurons (29,30), the present experimental results may indicate that, at least in part, the activation of estrogen receptors, with a certain range of the estradiol concentration, in modulating the neuronal bursting activities in the cultured hippocampal neurons is possibly directly modulating the potassium channel, particularly the Kv4.2 channel, properties by enhancing the channel current and channel-opening probability.

In conclusion, the present study identified that the estrogen level at a certain physiological concentration is important in maintaining the potassium channel activities and in turn influences the neuronal excitability to the extent of the epileptiform activities. Thus, the present results indicate that reduced activation of the transient outward potassium current by a high estrogen level may be one of the causes for triggering catamenial epilepsy, and provides us with a potential therapeutical target to intervene with catamenial epilepsy in the future.

Acknowledgements

The present study was supported by grants from the Nature Science Foundation of China (nos. 31129003, 81171224, 31271188 and 81301108) and the Science and Technology Commission of Shanghai Municipality (nos. 13DJ1400302 and 13ZR1406500) to YW., X.L. and XW.

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