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Letter to the Editor

Potentiation of 17β-estradiol on neuroexcitability by HCN-mediated neuromodulation of fast-afterhyperpolarization and late-afterdepolarization in low-threshold and sex-specific myelinated Ah-type baroreceptor neurons via GPR30 in female rats

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17β-Estradiol (17β-E_2), as a female hormone, demonstrates a boarder role in modulating neuroexcitability of visceral afferent neurons [1], such as baroreceptor neurons (BRNs), hence, participating in the baroreflex function [2], the cellular and ion channel mechanisms involved in this function have always attracted the keen attention of investigators. A wealth of information indicates that 17β-E_2 enhances the excitability in both CNS neurons [3] and visceral afferent neurons including BRNs [4] by modification of expression profiles of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels [5] underlying the funny current (I_f) or hyperpolarization-activated current (I_h), which is originally described in sinoatrial node myocytes as an inward current activated on hyperpolarization to the diastolic range of voltages and has the capability for generating repetitive activity [6]. The degree of activation of this current determines the steepness of phase 4 depolarization, the frequency of action potential firing and autonomic regulation of heart rate. In the visceral sensory system, the hyperpolarization-activated current (I_h) has also been observed mainly in myelinated afferents [4,7] and plays a key role in retaining the firing frequency of those neurons, whereas the neuroexcitability is reduced by ovariectomy [4] due at least partially to the downregulation of HCN1 in low threshold and sex-specific distribution of myelinated Ah-type afferents [8]. Our recent electrophysiological data have also shown that I_h mediates afterhyperpolarization and maintains the higher frequency of spike firing in Ah-type BRNs [1]. However, whether and how 17β-E_2 is involved in the regulation of neuroexcitability in Ah-type BRNs remained not elucidated.

To answer this question, BRNs were isolated from adult female rats and individually identified electrophysiologically and fluorescently [9,10]. Our results showed that, in the presence of 1.0 μM 17β-E_2, repetitive discharge evoked by step depolarization of Ah-type BRNs was significantly enhanced in a time-dependent fashion through all stimulus intensity applied (Fig. 1A–B). This effect of 17β-E_2 was mimicked by 100 nM G1, a selective agonist for G-protein-coupled receptor-30 (GRP30) [11], completely abolished by 100 nM G15, a selective antagonist for GPR30, and 300 nM pertussis toxin (PTX, Fig. 1C), suggesting that non-genomic and potentiated action of 17β-E_2 on neuroexcitability is due to G-protein coupled receptor activation. Consistent with previous observation [12], the effects of 17β-E_2 were not observed in either myelinated A-type or unmyelinated C-type BRNs.

To explore the underlying ion channel mechanisms of 17β-E_2's potentiation on respective discharge, large conductance Ca^{2+}-activated K^+ channel (KCa1.1) [13] and HCN1 [1] are the two major channels that control the neuroexcitability and that may also be involved somehow in the potentiation in the presence of 17β-E_2. Even though the functional downregulation of KCa1.1 in OVX females is responsible at least in part for the decreased neuroexcitability in Ah-type afferents neurons (data not shown), AP waveform seems not significantly altered before and after 17β-E_2, therefore, the involvement of KCa1.1 in 17β-E_2-mediated potentiation is largely excluded. Recent investigation has demonstrated that the downregulation of HCN1 expression is one of the plausible molecular mechanisms of less membrane excitation in myelinated Ah-type afferents by surgically removing ovaries [4]. We have also shown that discharge profile can be manipulated by fast

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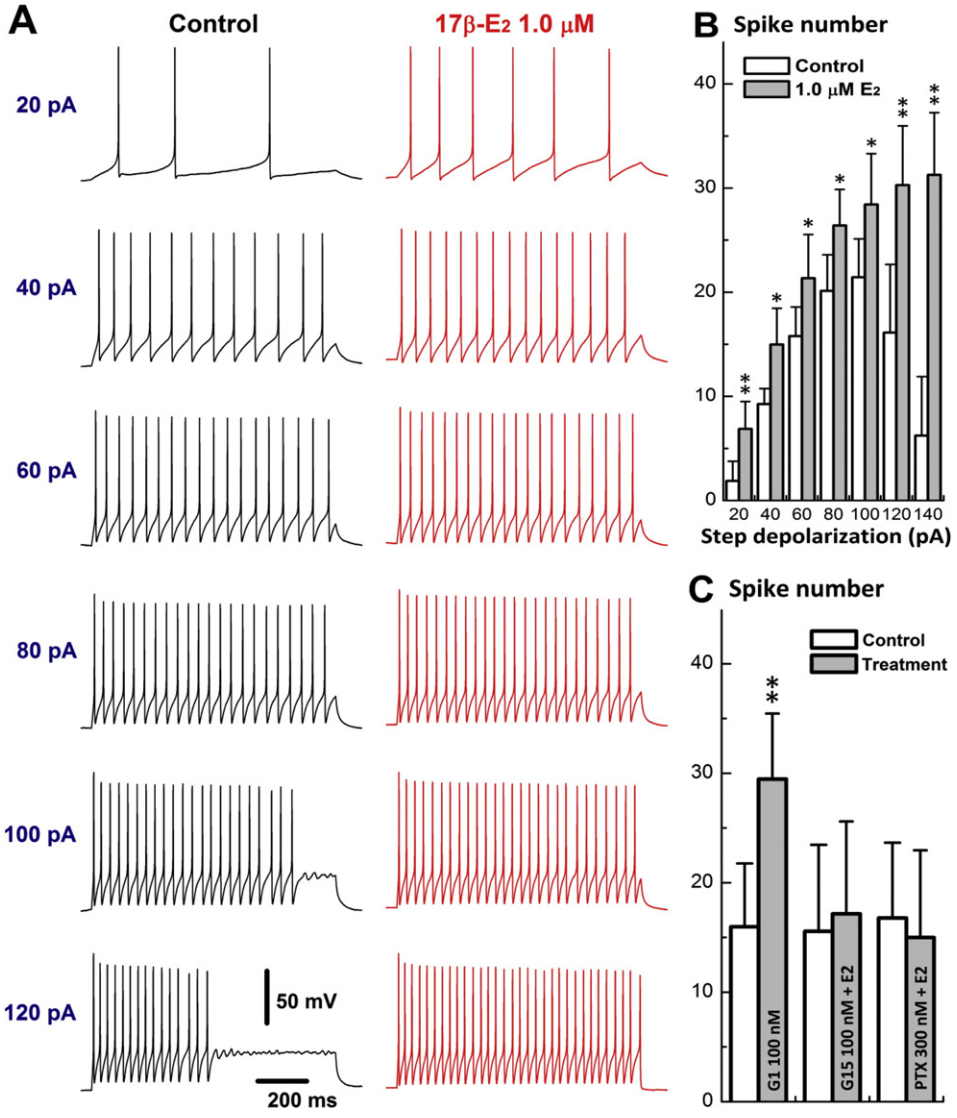

Fig. 1. 17β-estradiol (17β-E_2)-mediated potentiation of action potential (AP) repetitive firing elicited by step depolarization in low threshold and sex specific myelinated Ah-type baroreceptor neurons BRNs isolated from adult female rats. BRNs were firstly identified using electrophysiological standard validation and the spike trains were elicited by 1.0-s step depolarization from 20 pA to 140 pA in the presence of 1.0 μM 17β-E_2 ($n = 8$) at least for 40 min after administration. (A) The representatives of spike trains before and after 17β-E_2 under different step depolarizations, scale bars apply for all presented tracings; (B) Summarized data of 17β-E_2-mediated potentiation and the spike number was plotted as the functional of step depolarization currents; (C) Averaged data showing the effects of 100 nM G1 ($n = 7$), 100 nM G15 ($n = 6$), or 300 nM PTX ($n = 5$) on 17β-E_2's potentiation, respectively. 17β-E_2 was bath perfused after 5 min incubation of cells with G1, G15, or PTX and the recording was collected 10 min after 17β-E_2. The number of test recordings shown in each group was collected from at least 3 preparations. Averaged data were presented as mean ± SD, $^{*}P < 0.05$ and $^{**}P < 0.01$ vs control. A single AP was elicited by applying a brief (<500 μs) suprathreshold current pulse through the patch electrode. The depolarized current steps (1000 ms) were applied through the electrode from a reference potential of approximately −60 mV.

afterhyperpolarization (AHP_{fast}) via inactivation of HCN [1], suggesting that the hyperpolarization-activated current (I_h) is a potential target for further investigation. By carefully looking the AP trajectory, AHP_{fast} was less negative and the late afterdepolarization (ADP_{late}) was significant and occurred immediately after AHP_{fast} in the presence of 17β-E_2 (Fig. 2A–B) without affecting both depolarization and repolarization characters (Fig. 2C). Intriguingly, The ADP_{late} was enhanced dramatically in a time-dependent manner (Fig. 2D & inset, Table 1) and brief pulse with the same intensity eventually evoked burst spike with a progressively shorter spike interval along with the ADP_{late} reaching the threshold (Fig. 2E & inset). In another set of experiments, 17β-E_2 elicited larger depolarized voltage sag potential and rebound AP by termination of the hyperpolarization step compared with control and this result was consistent well with data collected with 100 nM G1 (Fig. 2F–H). These observations indicated that I_h mediated specific manipulations of AHP_{fast} and ADP_{late}, led the membrane potential rapidly and progressively shifting up to the AP firing threshold after the pulse-evoked first spike and induced spontaneous firing that is rarely seen in peripheral nerve, thereby resulting in 17β-E_2-dependent enhancement on neuroexcitability. Additionally, the resting membrane potential (RMP) was also significantly hyperpolarized (insets of Fig. 2D–E, Table 1) time-dependently with hyperpolarized shifting of AP firing threshold as well due presumably to increase in Na^+ channel availability at given voltage, which in turn drives the threshold closer to ADP_{late}, eventually resulting in the spontaneous AP spike after a pulse-evoked one in the presence of 17β-E_2, suggesting that HCN-mediated alternations in AHP_{fast}, ADP_{late}, and RMP/threshold were synchronously and synergistically modulated by 17β-E_2.

In order to further confirm the involvement of HCN channel in 17β-E_2-induced discharge potentiation, the I_h was recorded with or without 17β-E_2. Consistent with current-clamp data, 1.0 μM 17β-E_2 enhanced I_h density (Fig. 3A–B, E) and the voltage-dependent activation was rightward shifted without changing the slope (Fig. 3F, Table 2). The increasing evidence demonstrates that the frequency-dependent activation