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Estrogen modulates potassium currents and expression of the Kv4.2 subunit in GT1-7 cells☆

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Abstract

The proper maintenance of reproduction requires the pulsatile secretion of gonadotropin-releasing hormone (GnRH), which is ensured by synchronized periodic firing of multiple GnRH neurons. Both hormone secretion and electrophysiological properties of GnRH cells are influenced by estrogen. The impact of 17β-estradiol treatment on the function of voltage gated A- and K-type potassium channels, known modulators of firing rate, was therefore examined in our experiments using immortalized GnRH-producing GT1-7 neurons. Whole cell patch clamp recordings showed the absence of the A-type current in GT1-7 cells cultured in estrogen-free medium and after 8 h 17β-estradiol treatment. Exposure of the cells to 17β-estradiol for 24 and 48 h, respectively, resulted in the appearance of the A-type current. The induction of the A-type current by 17β-estradiol was dose-related (50 pM to 15 nM range). In contrast, the K-type potassium current was apparent in the estrogen-free environment and 17β-estradiol administration significantly decreased its amplitude. Co-administration of 17β-estradiol and estrogen receptor blocker, Faslodex (ICI 182,780; 1 μM) abolished the occurrence of the A-type current. Real-time PCR data demonstrated that expression of the Kv4.2 subunit of the A-type channel was low at 0, 0.5, 2 and 8 h, peaked at 24 h and diminished at 48 h 17β-estradiol treatment (15 nM).

These data indicate that potassium channels of GT1-7 neurons are regulated by estrogen a mechanism that might contribute to modulation of firing rate and hormone secretion in GnRH neurons.

Keywords: Estrogen; Potassium current; Hypothalamus; GnRH neurons; GT1-7

1. Introduction

In the hypothalamus, gonadotropin-releasing hormone (GnRH) plays an important role in the control of fertility of vertebrates. In the pituitary gland, it induces synthesis and release of the luteinizing hormone (LH) and follicle stimulating hormone (FSH) which subsequently act on the gonads and control reproductive functions such as maturation of gametes, the onset of puberty and the ovulatory cycle. GnRH is released in a pulsatile manner (Levine and Ramirez, 1982; Levine, 1999) and changes in the frequency of this release influences secretion of both LH and FSH (Wildt et al., 1981) and consequently, the estrous cycle and fertility. GnRH secretion is dependent on the intracellular Ca^{2+} content (Martinez de la Escalera et al., 1992) and the presence of the action potentials (Mellon et al., 1990). Pulsatility and volume of the secretion can, therefore, be correlated with the synchronized firing rate of the GnRH-producing cells (Kato et al., 1994; Wada et al., 2006).

One of the hormones altering GnRH pulse frequency is estrogen that can evoke both positive and negative feedback effects depending on the phase of the estrus cycle (Ordog and Knobil, 1995). In accordance with its effect on the GnRH secretion, estrogen alters the firing rate of the GnRH-producing neurons. It has been shown to increase the firing frequency and burst duration of primary cultured LHRH-1 neurons of the primate brain (Abe and Terasawa, 2005). In contrast, a decreased neuronal firing was demonstrated in the GnRH producing neurons recorded in brain slices taken from ovariectomized, estrogen substituted mice (Christian et al., 2005).

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Frequency of the action potential is highly regulated by the voltage-gated potassium (Kv) channels such as the rapidly inactivating A-type and the delayed rectifier K-type (Hille, 1994). These channels shape the action potential by controlling its repolarization phase and determine the membrane potential and duration of the interspike interval. The K-type channels keep single action potentials short and permit high frequency trains of them. The A-type channels ensure low frequency firing of cells and promote broadening of the action potentials during repetitive activity (Birnbaum et al., 2004). The functional properties of the voltage gated potassium channels are determined by their α- and β-subunits. Most Kvα subunits encode delayed rectifier channels with slow inactivation whereas few of them, such as Kv4.2 contribute to the function of the A-type channels. The potassium channels are known to control survival of neurons, hormone secretion, and neurotransmitter release in the CNS (Neuchs et al., 2003).

Estrogen affects function of various potassium channels in the brain. It has been shown to inhibit the activity of the outward potassium channels in the rat parabrachial nucleus (Fatehi et al., 2006) and modulate the small conductance calcium-activated SK3 potassium channels in the hypothalamus of the female guinea pig (Bosch et al., 2002). Hence, we hypothesized that the estrogen-evoked decrease in the firing rate of the GnRH neurons might be mediated, in part, via the alteration of potassium channels. Therefore, estrogen-dependency of the potassium currents was analyzed in GT1-7 neurons, cells of an immortalized GnRH-producing cell line (Mellon et al., 1990). This cell line has been shown to synthesize GnRH and secrete it in a pulsatile manner (Wetsel et al., 1992; Martinez de la Escalera et al., 1992). The electrical activity of the GT1-7 neuronal network is synchronized and they fire with bursts of action potentials (Hiruma et al., 1997; Nunemaker et al., 2001; Funabashi et al., 2001; Martinez-Fuentes et al., 2004). Furthermore they contain estrogen receptor-α and β (ERα and ERβ) (Lopez et al., 1996; Roy et al., 1999; Kallo et al., 2001), and are highly responsive to estrogen (Morales et al., 2003). In addition, GnRH-producing cell lines such as GT1-1 and GT1-7 express A- and K-type potassium channels (Bosma, 1993; Constantin and Charles, 2001).

In the present study, the influence of estrogen treatment on the parameters of the A- and K-type potassium channels of GT1-7 neurons was examined by whole cell clamp electrophysiology, and the putative estrogen-dependent expression of the Kv4.2 mRNA, encoding in one of the subunits of the A-type potassium channel was explored by means of real time PCR method.

2. Materials and methods

2.1. Cell culture

GT1-7 cells were cultured in Dulbecco Modified Eagle Medium (DMEM; SIGMA) containing high-glucose and supplemented with 10% fetal calf serum (FCS; HyClone) and 5% horse serum (HS; HyClone). Before 17β-estradiol treatment, the culturing medium was replaced to a steroid/thyroid- and phenol red-free DMEM supplemented with serum replacement 2 (SR-2) free of estrogen and other steroid and thyroid factors (DMEM-SR2; SIGMA), and the cells were cultured in this solution for 48 h. Water-soluble 17β-estradiol (E2; SIGMA) was then added in a concentration of 10 nM for PCR and 50 pM to 15 nM for patch clamp experiments for different duration. The drug concentration administered was in accordance with the concentration used by other authors (Carrer et al., 2003; Morales et al., 2003; Navarro et al., 2003; Nunez and McCarthy, 2003; van de Stolpe et al., 2004; Matagne et al., 2005; Pak et al., 2006). The control cells were used after the period of 48 h culturing in the E2-free medium. In order to determine whether the observed effects were due to the E2 administration, before administering the E2 one group of the cells was pretreated in the DMEM-SR2 medium for 30 min with Faslodex (ICI 182,780; 1 μM; Tocris Inc.), a blocker of both of the estrogen receptors α and β. E2 was then added and the neurons were co-treated with the E2 + Faslodex + DMEM-SR2 mixture for 24 h. For control measurements, 0.001% DMSO (dissolvent of Faslodex) was used as a vehicle instead of Faslodex.

2.2. Reverse transcription

Total RNA from control and E2 treated GT1-7 cells was isolated with TRIzol LS reagent (Invitrogen) according to the manufacturers' instructions. RNA from three equivalent cultures were mixed and the RNA solutions were diluted to reach a final concentration of 1 μg/μl. From each treatment group 2 μg total RNA was used for cDNA synthesis. cDNA reaction mixtures (40 μl) contained oligodT, random hexamers and 1.5 mM $MgCl_2$. Reverse transcription was performed with the ImProm II Reverse Transcription System (Promega) in a Perkin-Elmer thermal cycler using the method described by the manufacturer.

2.3. Real-time quantitative PCR

Real-time polymerase chain reactions (PCR) were carried out in a Light Cycler PCR machine (Roche) with the DNA Master SYBR Green I mix (Roche) according to the manufacturers protocols in 10 μl reaction volumes using Light Cycler glass capillaries (Roche). PCR reactions contained 1 μl DNA Master SYBR Green I (Roche), 1 μl cDNA mix, 4 mM $MgCl_2$, 0.3 μM specific primers and were run three times. Hypoxanthine-guanine phosphoribosil transferase (HPRT) gene was used as a house keeping gene that is not regulated by estrogen and the differences in the amounts of the HPRT mRNA between treatment groups were used to calculate the amounts of the Kv4.2 potassium channel subunit. The standard curves were created by amplifications of oligonucleotides containing partial sequences of the HPRT and Kv4.2 genes with the same primer sequences as the products were amplified. The oligonucleotide standards were diluted 10-fold in TE buffer over a range spanning the sample concentrations. For quantification, the test oligonucleotide was applied in a dilution close to the sample concentrations. The reverse transcribed cDNA were from samples collected at 0, 0.5, 2, 8, 24 and 48 h after E2 treatment. H_2O was included as no template control. Real-time PCR conditions were as follows: for the HPRT gene 95 °C 5 min for denaturation and 65 cycles for 94 °C 5 s, 57 °C 7 s and 72 °C 10 s; for the Kv4.2 gene 95 °C 30 s and 65 cycles for 94 °C 5 s, 61 °C 7 s and 72 °C 10 s and cooling to 40 °C took 30 s. Primer sequences used in the PCR reactions were as follows: HPRT forward 5′-tgt aat gat cag tca acg ggg-3′, reverse 5′-tgg cct gta tcc aac act tcg-3′; Kv4.2 forward 5′-agg cgg gag aat gct gag cgc ct-3′, reverse 5′-ggc cac cac gtc gat gat act cat-3′.

The relative amount of the products was determined from the log phase of the reaction. The quantitative results were expressed as the ratio comparing to the highest dilution of the HPRT.

2.4. Whole cell clamp experiments

The cells were cultured in a steroid-free medium for 48 h period then four groups of the GT1-7 neurons were established. The first, control group received no further treatment; the second group was treated with various concentrations (50, 150, 450 pM, 1.5, 4.5, 15 nM) of E2 for 24 h or with 15 nM E2 for various time (8, 24, 48 h); the third one with Faslodex + E2 (24 h) and the fourth one also for control purposes with 0.001% DMSO + E2 (DMSO was used as solvent of the Faslodex) for 24 h. The GT1-7 cells were voltage clamped at room temperature using a whole-cell clamp configuration. The instruments used for electrophysiology were as follows: Axopatch 200-A patch clamp amplifier, Digidata-1200 data acquisition system and pCLAMP 6.02 software from Axon