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Brain Research

Research Report

Estradiol rapidly modulates synaptic plasticity of hippocampal neurons: Involvement of kinase networks

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ARTICLE INFO

Article history:
Accepted 27 December 2014
Available online 13 January 2015

Keywords:
Estradiol
Estrogen
Estrogen receptor
LTP
Spine
Synaptic plasticity
Kinase
Rapid action

ABSTRACT

Estradiol (E2) is locally synthesized within the hippocampus in addition to the gonads. Rapid modulation of hippocampal synaptic plasticity by E2 is essential for synaptic regulation. Molecular mechanisms of modulation through synaptic estrogen receptor (ER) and its downstream signaling, however, have been still unknown.

We investigated induction of LTP by the presence of E2 upon weak theta burst stimulation (weak-TBS) in CA1 region of adult male hippocampus. Since only weak-TBS did not induce full-LTP, weak-TBS was sub-threshold stimulation. We observed LTP induction by the presence of E2, after incubation of hippocampal slices with 10 nM E2 for 30 min, upon weak-TBS. This E2-induced LTP was blocked by ICI, an ER antagonist. This E2-LTP induction was inhibited by blocking Erk MAPK, PKA, PKC, PI3K, NR2B and CaMKII, individually, suggesting that Erk MAPK, PKA, PKC, PI3K and CaMKII may be involved in downstream signaling for activation of NMDA receptors. Interestingly, dihydrotestosterone suppressed the E2-LTP.

We also investigated rapid changes of dendritic spines (=postsynapses) in response to E2, using hippocampal slices from adult male rats. We found 1 nM E2 increased the density of spines by approximately 1.3-fold within 2 h by imaging Lucifer Yellow-injected CA1 pyramidal neurons. The E2-induced spine increase was blocked by ICI. The increase in

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http://dx.doi.org/10.1016/j.brainres.2014.12.056
0006-8993/

spines was suppressed by blocking PI3K, Erk MAPK, p38 MAPK, PKA, PKC, LIMK, CaMKII or calcineurin, individually. On the other hand, blocking JNK did not inhibit the E2-induced spine increase.

Taken together, these results suggest that E2 rapidly induced LTP and also increased the spine density through kinase networks that are driven by synaptic ER.

This article is part of a Special Issue entitled SI: Brain and Memory.

1. Introduction

Finding of local synthesis of estrogen and androgen in the adult male/female hippocampus opened a new field of estrogen function in relation to the regulation of daily memory formation (Kimoto et al., 2001; Kawato et al., 2002, 2003; Hojo et al., 2004; Kretz et al., 2004; Hojo et al., 2008; Okamoto et al., 2012; Kato et al., 2013). The level of adult male hippocampal estradiol (E2) is higher (~8 nM) than that of plasma E2 (~0.01 nM), as determined by mass-spectrometric analysis (Hojo et al., 2009). Therefore, it is important to investigate modulation effects by hippocampal E2 on synaptic plasticity.

In addition to slow modulation effects on synaptic plasticity by circulating E2, E2 exerts rapid (1–2 h) influence on the synaptic plasticity of rat hippocampal glutamatergic neurons in slices, as has been demonstrated by a number of electrophysiological investigations in rats and mice, concerning the long-term potentiation (LTP) in CA1 (Foy et al. 1999; Bi et al., 2000), the long-term depression (LTD) in CA1 (Vouimba et al., 2000; Mukai et al., 2007) and kainate current in CA1 (Gu and Moss, 1996; Gu et al., 1999).

The rapid effect of E2 may be driven through either ERα or ERβ, possibly localized at the membrane, in analogy with cultured cells of peripheral origin (McEwen and Alves, 1999; Razandi et al., 1999). It is probable that synaptically localized ERα or ERβ (Milner et al., 2005; Mukai et al., 2007, 2010; Hojo et al., 2008) may participate in rapid modulation by E2. Involvement of non-ERα or non-ERβ type receptor, however, might not be excluded (Gu and Moss, 1996; Gu et al., 1999).

On the other hand, extensive studies have been performed to investigate the role of E2 in slowly (1–4 days) modulating hippocampal plasticity, because the hippocampus is known to be a target for the actions of gonadal estrogens reaching the brain via blood circulation. For example, the density of dendritic spines in the CA1 pyramidal neurons is modulated *in vivo* by supplement of E2 in ovariectomized (OVX) animals (Gould et al., 1990; Woolley et al., 1990; Woolley and McEwen, 1992; Leranth et al., 2000, 2002), resulting in increase/recovery the number of spines. *In vitro* investigations have also shown that spine density is increased following several days' treatment of cultured hippocampal slices with E2 (Murphy and Segal, 1996; Pozzo-Miller et al., 1999). Other type of E2 effects on synaptic receptors of the hippocampal neurons has also been accumulated, including an NMDA receptor-dependent mechanism of E2 regulation on dendritic spine density (Woolley and McEwen, 1994), and increase of glutamate binding to NMDA receptors by E2 (Woolley et al., 1997). LTP study of slow/genomic effects shows E2-induced enhancement of LTP after 24–48 h of E2 injection to OVX rats (Smith et al., 2002).

The current study was designed, using acute hippocampal slices from adult male rats, to investigate (1) kinase dependence of signaling mechanisms in rapid modulation of E2-induced LTP upon weak theta burst stimulation (weak-TBS) and (2) kinase dependence of E2-induced rapid spinogenesis. Selective blockers of many kinases were used. In order to investigate E2-induced LTP, slices were incubated with E2, and weak-TBS was applied. These weak-TBS methods were employed, because E2 application does not enhance tetanus-induced LTP in 3 month-old adult rat hippocampus (Ooishi et al., 2012b), since strong tetanus probably saturates LTP.

Fig. 1 – E2 induces LTP upon weak-TBS in the CA1 hippocampal slices. (A1) Slices with 0 nM E2 (Cont) (closed circle, $n=10$ slices), with 10 nM E2 (E2) (open circle, $n=10$ slices), with 10 nM E2 plus 100 nM ICI (E2+ICI) (open triangle, $n=8$ slices). (A2) Slices with 0 nM E2 (Cont), with 100 nM PPT (PPT) (open square, $n=8$ slices), with 100 nM WAY-200070 (WAY) (closed triangle, $n=8$ slices). Vertical axis indicates EPSP slope. Here, 100% refers to the EPSP slope value of the average of $t=-9$ to 0 min prior to weak-TBS stimulation. LTP was induced at time $t=0$. Illustrated data points and error bars represent the mean ± SEM from n of independent slices. (B1) Effect of kinase inhibitors. Co-incubation of E2 with PKA inhibitor H89 (10 μM) prevented the induction of LTP (E2+H89) (open square, $n=8$ slices), with MAPK inhibitor U0126 (10 μM) prevented the induction of LTP (E2+U) (closed circle, $n=8$ slices), with PKC inhibitor chelerythrine (10 μM) prevented the induction of LTP (E2+Chel) (open triangle, $n=8$ slices). (B2) Co-incubation of E2 with mGluR1 inhibitor YM202074 (4 μM) did not prevent the induction of LTP (E2+YM) (closed triangle, $n=8$ slices). (C1) NR2B inhibitor Ro25-6981 (1 μM) suppressed the E2-LTP (E2+Ro) (closed triangle, $n=8$ slices). (C2) CaMKII inhibitor KN-93 (20 μM) suppressed the E2-LTP (E2+KN) (open square, $n=8$ slices). Perfusion of only KN (20 μM) did not change the induction of the small LTP upon weak-TBS (KN) (closed square, $n=5$ slices). (D) Comparison of inhibitor-induced modulation effects on E2-LTP as shown in (A), (B) and (C) Light most is full-LTP induced by full-TBS (full-TBS). Statistical significance was defined as $^{*}p<0.05$, $^{**}p<0.01$. (E) Representative raw traces of EPSP, showing sample recordings prior to (black line) or after (gray line) weak-TBS stimulation.