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Effects of 17β-estradiol on chemically induced long-term depression

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Abstract

In this study, we have investigated the effects of 17β-estradiol (E2) on chemically induced long-term depression (LTD). LTD was induced by a brief application of *N*-methyl-D-aspartate (NMDA) or (*R,S*)-3,5-dihydroxyphenylglycine (DHPG), a group I metabotropic glutamate receptor agonist. Bath application of E2 alone potentiated population excitatory postsynaptic potentials. This potentiation was readily reversed by washing with control saline. The effect of E2 on NMDA-induced LTD was a conversion of LTD to long-term potentiation (LTP). An application of NMDA in the presence of E2 induced LTP. The induction of LTP was inhibited by an inhibitor of calcium/calmodulin dependent protein kinase (CaMKII). The results suggest that E2 potentiates NMDA receptor function and induces an increase in postsynaptic Ca^{2+} concentration. An increase in postsynaptic Ca^{2+} concentration activates CaMKII, leading to LTP. In contrast to NMDA-induced LTD, an application of DHPG in the presence of E2 induced significantly larger LTD. The results suggest that E2 potentiates an as yet unidentified process(es) in inducing LTD by an application of DHPG. The effects of E2 both on NMDA-induced and DHPG-induced LTD were suppressed by an estrogen receptor antagonist.

Keywords: Synaptic plasticity; Long-term depression (LTD); Hippocampus; Estradiol; (*R,S*)-3,5-Dihydroxyphenylglycine (DHPG); *N*-Methyl-D-aspartate (NMDA)

1. Introduction

It has been reported that the female steroid hormone 17β-estradiol (E2) modulates synaptic plasticity in the hippocampus. Acute application of E2 increases basal synaptic responses and the magnitude of long-term potentiation (LTP) (Foy et al., 1999; Bi et al., 2000; Kim et al., 2002). The increase in the magnitude of LTP could be due to increases in *N*-methyl-D-aspartate (NMDA) receptor mediated responses (Foy et al., 1999).

Two different results have been reported on the effects of E2 on long-term depression (LTD). One is the report by Vouimba et al. (2000) and the other is by Desmond et al. (2000). Vouimba et al. have reported that E2 suppressed the induction of LTD in aged rats and produced only a minimal effect in suppressing LTD in adult rats. On the other hand, Desmond et al. (2000) have shown that E2 enhances LTD induction of the adult rat hippocampus. Synaptically-induced LTD can be either NMDA receptor-dependent (Dudek and Bear, 1992; Mulkey and Malenka, 1992) or metabotropic glutamate (mGlu) receptor-dependent (Bashir et al., 1993), and both forms can co-exist at the same synapses depending on the experimental conditions (Oliet et al., 1997).

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The mechanisms of induction and expression of these two types of LTD are dependent on age and stimulus protocols (Kemp et al., 2000). NMDA receptor-dependent LTD is induced by delivering a prolonged period of low frequency stimulation (LFS), and mGlu receptor-dependent LTD by delivering a similarly prolonged period of paired-pulse stimulation (PP-LFS) (Kemp and Bashir, 2001). Vouimba et al. (2000) induced LTD by LFS and Desmond et al. (2000) by asynchronous LFS. Thus, the discrepancy between the two reports by Vouimba et al. and Desmond et al. may be attributed to the relative importance of NMDA receptors and mGlu receptors in inducing LTD.

The purpose of the present study is to evaluate the effects of E2 on LTD induced by NMDA or mGlu receptors activation separately. To do this we utilized the findings that pharmacological activation of NMDA (Lee et al., 1998) or mGlu (Palmer et al., 1997) receptors can induce LTD. The effects of E2 were investigated on LTD induced by a brief application of NMDA (NMDA-induced LTD) and induced by a brief application of a group I mGlu receptor specific agonist (*R,S*)-3,5-dihydroxyphenylglycine (DHPG-induced LTD).

2. Materials and method

2.1. Preparation of hippocampal slices

Slices were prepared as described previously (Natsume and Kometani, 1997). Briefly, male Sprague–Dawley rats (150–200 g) were decapitated under deep ether anesthesia. The brain was removed quickly and placed in ice-cold, oxygenated (95% O_2–5% CO_2) Krebs solution (in mM: 124 NaCl, 5 KCl, 1.24 KH_2PO_4, 1.3 $MgSO_4$, 2.4 $CaCl_2$, 26 $NaHCO_3$, 10 glucose). Transverse slices (500–550 μm thick) were cut with a micro tissue slicer (YI-S102, ASTEC) and were placed for at least 1 h on a nylon mesh screen in a tissue chamber filled with oxygenated Krebs solution at 33 °C. A single slice was transferred to a thermostatted (33±0.5 °C) interface recording chamber (Nakazawa Seisakusyo) where it was superfused with oxygenated Krebs solution at 33 °C at a rate of 1.5–2 ml/min. The upper surface of the slice was exposed to an atmosphere of humidified and warmed 95% O_2–5% CO_2.

2.2. Electrophysiological recording

Population EPSPs (pEPSPs) were recorded with a glass microelectrode (2–5 MΩ) placed in the stratum radiatum of CA1. The EPSP was elicited via stimulation of the stratum radiatum with a bipolar tungsten electrode. The intensity of a 30 s interval test stimulus was adjusted to elicit pEPSP with an amplitude 50% of the maximum. After checking the stability of the responses, the perfusate was switched to the NMDA (20 μM) or DHPG (50 μM) containing solution. After a brief application of the drug, the perfusate was changed to the control Krebs solution and the responses to test stimuli were observed for at least 45 min. The magnitude of NMDA-induced LTD or DHPG-induced LTD was evaluated by the ratio of the averaged pEPSP for 40–45 min after to that for 10 min before the drug application. The effects of E2 on NMDA- or DHPG-induced LTD were investigated by an application of NMDA or DHPG in the presence of E2 (1 nM).

LTD induction by DHPG and the effects of E2 on DHPG-induced LTD were investigated with slices taken from the same rat. This was necessary to insure that the effects of E2 on LTD are not simply due to day to day variability in LTD induction.

Signals from the microelectrode were amplified, filtered (<10 kHz) using an amplifier (Nihon Koden MEZ-7200) and sampled with a personal computer (NEC PC-9801 VX). All values are expressed as means±SEM. Comparisons between two means were analyzed by Student's *t*-test, and probabilities less than 0.05 were considered significant.

DHPG and 7α-[9-[(4,4,5,5,5,-pentafluoropentyl)sulfinyl]nonyl]-estra-1,3,5(10)-triene-3,17β-diol (ICI 182,780) were purchased from Tocris. NMDA, E2 and 1-[*N,O*-bis-(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine (KN-62) were purchased from Sigma and all other chemicals were from Wako.

3. Results

3.1. NMDA- and DHPG-induced LTD

Fig. 1a shows an averaged time course of NMDA-induced LTD. A 3 min application of 20 μM NMDA reduced pEPSPs transiently. The pEPSPs then gradually recovered to a level lower than the control. The time course is similar to that reported by Lee et al. (1998). The magnitude of NMDA-induced LTD was 0.76±0.04 ($n = 7$).

A time course of DHPG-induced LTD shown in Fig. 1b was similar to that of NMDA-induced LTD. After a transient reduction during a 5 min application of 50 μM DHPG, pEPSPs slowly increased and reached a stable amplitude. The magnitude of DHPG-induced LTD was 0.71±0.05 ($n = 7$).

3.2. Effects of E2 on pEPSPs and chemically induced LTD

Fig. 2a shows an averaged time course of results obtained from experiments investigating the effect of E2 on pEPSPs. The figure shows that E2 induces a rapid and reversible potentiation of pEPSPs. During E2 application