Bisphenol A significantly modulates long-term depression in the hippocampus as observed by multi-electrode system

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Abstract

OBJECTIVE: Low dose exposure to endocrine disrupters (environmental chemicals) may induce hormone-like effects on wildlife and humans. bisphenol A (BPA) might disturb the neuronal signaling regulated by endogenous estrogens. We investigated the rapid modulation effects of 10nM BPA, a typical endocrine disruptor, on long-term depression (LTD) of adult rat hippocampal slices.
METHOD: LTD was induced by a transient perfusion of 30 μM NMDA for 3 min. And measured with multielectrode probes.
RESULT: A 30 min perfusion of 10 nM BPA rapidly enhanced LTD in CA1, however, BPA suppressed LTD in dentate gyrus (DG). An ERRγ antagonist, 4-OH-tamoxifen, suppressed LTD in CA1 and DG. Inhibitor of estrogen receptor ICI 182,780 did not disturb BPA effects. On the other hand, tributyltin (TBT), another endocrine disruptor, did not have any effect on LTD in CA1 and DG.
CONCLUSION: ERRγ, but not estrogen receptors, is a high affinity BPA receptor in LTD processes, since the effect of BPA on LTD was suppressed by an ERRγ antagonist. A possible mechanisms of BPA-induced enhancement of LTD could be described with ERRγ, MAPK activation and phosphorylation of MMDA receptors.

ORIGINAL ARTICLE

INTRODUCTION

Low dose exposure to endocrine disrupters (environmental chemicals) may induce hormone-like effects on wildlife and humans. Bisphenol A (BPA) is a widely used synthetic material included in polycarbonate resin used in water pipe sealant, dental prostheses, compact discs and baby bottles. The low dose exposure to BPA (μg/kg/day or nanomolar doses) shows rather weak toxic effects on reproductive or endocrine functions in the peripheral tissues, however, the effects of low dose exposure to BPA during fetal/neonatal stages have been extensively investigated. For example, fetal or neonatal exposure to BPA inhibits sexual differentiation of nonreproductive behaviors of adult animals, including maze learning behavior (Carr *et al.* 2003; Kubo *et al.* 2003; Fujimoto *et al.* 2006), at doses as low as 1/1,000 of those required for the stimulation of uterine growth (Ashby, 2001).

High dose tributyltin (TBT) (antifouling agents in fishnets and marine paints for ships) (5 μM) has induced severe neuronal death in a concentration- and time-dependent manner in organotypic slice culture s of immature rat hippocampus (Mizuhashi *et al.* 2000). Toxic effects of high dose (mg/kg body) TBT have also been investigated about development and functions of the reproduction systems (Fisher *et al.* 1999; Al-Hiyasat *et al.* 2002; Grote *et al.* 2004; Halldin *et al.* 2005). Peripubertal exposure to 15 mg TBT/kg considerably affects male sexual development (Grote *et al.* 2004). However, the low dose (μg/kg) exposure to TBT has not been reported to induce significant effects on reproductive or endocrine functions in the peripheral organs, probably due to efficient detoxification of chemicals in the liver.

In the hippocampus, hippocampal-synthesized estradiol (E2) (Kawato *et al.* 2002; Hojo *et al.* 2004; Kretz *et al.* 2004; Hojo *et al.* 2009) and/or circulating E2 play a significant role in the modulation of synaptic transmission, including long-term depression (LTD) and long-term potentiation (LTP) (Foy *et al.* 1999; Mukai *et al.* 2006; Mukai *et al.* 2007; Mukai *et al.* 2010). BPA or TBT might disturb the neuronal signaling regulated by endogenous estrogens. The slow and genomic effects of BPA or TBT on neuronal development, sexual differentiation and neuronal protection have been investigated (Sato *et al.* 2002; Kaitsuka *et al.* 2007; Zhang *et al.* 2008; Vandenberg *et al.* 2009). On the other hand, almost no investigations on rapid effects of BPA or TBT on LTD have been conducted in hippocampal neurons, except for our preliminary studies (Kawato, 2004; Ogiue-Ikeda *et al.* 2008).

One of most important problems of endocrine disrupters is the difficulty in identification of their binding sites/proteins. We have recently demonstrated the rapid effects of BPA on spinogenesis in hippocampal neurons via estrogen receptor-related receptor gamma (ERRγ) (Tanabe *et al.* 2012). Here, we show the actions of BPA in the adult hippocampal slices by measuring rapid modulation of N-methyl-D-aspartate (NMDA)-induced LTD simultaneously in CA1 and dentate gyrus (DG), by using the custom-made multi-electrode system, which could be a promising high-sensitive detection system of endocrine disrupters that affect the synaptic plasticity.

MATERIALS AND METHODS

Chemicals

BPA, ICI 182,780 and NMDA were purchased from Sigma (USA). Tributyltin (TBT) was from Tokyo Chemical Industry (Japan). 4-OH-tamoxifen was from Calbiochem (Germany). All other chemicals were of highest purity commercially available.

Animals

Adult male Wistar rats (12 weeks old, 340–360 g) were purchased from Saitama Experimental Animal Supply (Japan). The experimental procedure of this research was approved by the Committee for Animal Research of the University of Tokyo.

Preparation of hippocampal slices

Hippocampal slices were prepared as described previously (Mukai *et al.* 2007). Briefly, rats were deeply anesthetized and decapitated. The brains from rats were collected and placed in artificial cerebrospinal fluid (ACSF) at 4 °C. The dorsal hippocampus was dissected and 300 μm-thick slices transverse to the long axis, from the middle third of the hippocampus, were prepared with a vibratome slicer (Dosaka, Japan). ACSF was consisted of (mM): 124 NaCl, 5.0 KCl, 1.25 NaH_2PO_4, 2.0 $MgSO_4$, 2.0 $CaCl_2$, 22 $NaHCO_3$ and 10 glucose; and was equilibrated with 95% O_2/5% CO_2. Slices were used for the electrophysiological measurements after 2 hr recovery incubation in 95% O_2/5% CO_2-gassed ACSF at 25 °C.

LTD measurements with custom multi-electrode probes

MED64 multielectrode apparatus (Panasonic, Japan) was used for the electrophysiological measurements (Mukai *et al.* 2007). Hippocampal slices were positioned on a custom-made multielectrode probe in which 64 planar microelectrodes were arranged to densely cover the stratum radiatum in CA1, the stratum radiatum in CA3, and the molecular layer in DG (Supplementary Fig. S1). Bipolar constant current pulses (approx. 60 μA, 0.1 ms) served as a test stimulus at three selected electrodes as shown in Supplementary Figure S1.

Electrical signals from the remaining 61 electrodes were simultaneously measured, filtered through a 1 Hz–10 kHz band-pass filter and recorded at a 20 kHz sampling rate. Three of 61 recording electrodes were selected for the analysis of the field excitatory postsynaptic potential (EPSP) responses in CA1 and DG.

BPA (10 and 100 nM) and TBT (100 nM) were perfused (at 30 °C with perfusion rate of 2 ml/min) from 30 min before the induction of LTD which was induced by

a transient perfusion of 30 μM NMDA for 3 min (Lee *et al.* 1998). It should be noted that we employed the chemically-induced LTD because it is very difficult to induce LTD electrically in adult hippocampus. NMDA-LTD and electrically-induced LTD are likely to be generated by the similar induction mechanism in CA1 glutamatergic neurons (Lee *et al.* 1998). A hippocampal slice from the same animal was used for control experiments without endocrine disrupters.

Statistical analysis

Data are expressed as mean ± SEM. The significance of drug effect was examined via statistical analysis using Tukey-Kramer post-hoc multiple comparisons test when one-way ANOVA tests yielded $p<0.05$.

RESULTS

Electrophysiological recordings were performed by using novel 64 multielectrodes (Supplementary Figure S1). The acute effects of BPA and TBT on synaptic transmission were investigated simultaneously in two regions (CA1 and DG) of the same adult hippocampal slices. Before performing experiments, it was not known which regions were more sensitive to endocrine disrupters, since CA1 and DG have different types of neurons, synapse formations and LTD mechanisms. LTD was induced pharmacologically by a transient application (3min) of NMDA (Lee *et al.* 1998).

Upon application of 30 μM NMDA for 3 min to control slices, the maximal amplitude of EPSP was transiently decreased to a minimal value and then recovered to a plateau level within 30–50 min after the NMDA application. The plateau EPSP amplitude at 60 min was 82.3 % (CA1), and 95.0 % (DG), respectively (Figure 1A).

A 30 min preperfusion of 10 nM BPA significantly enhanced LTD in CA1 resulting in the EPSP amplitude at 60 min of 72.3 % (CA1) (Figure 1A). On the other hand, 10 nM BPA significantly suppressed LTD in DG, resulting in the EPSP amplitude of 100.5 % (DG). By increasing the concentration of BPA to 100 nM, BPA further enhanced LTD in CA1 (68.6 %), however BPA had no effect on LTD in DG (Figure 1A). An ERRγ antagonist, 4-OH-tamoxifen, suppressed the BPA-induced modulation of LTD in CA1 and DG (Figure 1B). On the other hand, an antagonist of ERα/ERβ, ICI 182,780, did not suppress the modulation of LTD by BPA.

Note that no significant increase in baseline EPSP signals was observed by the application of endocrine disrupters alone within 30–60 min prior to NMDA stimulation.

To show results are summarized in Figure 1 (B), in which a quantitative comparison was shown by normalizing the EPSP amplitude of the control LTD at 60 min to 100%. This normalization was performed for all pairs of control and drug-treated slices, because the control LTD responses varied slightly between different drug-treatment pairs, depending on differences in animals. BPA enhanced LTD in CA1. On the other hand, In DG, BPA induced no change in LTD.

DISCUSSION

We demonstrated here the rapid effects of BPA and TBT on the hippocampal LTD (Figure 1). The enhancing effects by BPA on LTD in CA1 were similar to those by E2 (Mukai *et al.* 2007), but effects by BPA in DG were different from those by E2 (Supplementary Figure S2) (Mukai *et al.* 2007). On the other hand, effects of TBT on LTD in CA1 are very different from those of BPA or E2. TBT did not induce any effect on LTD in CA1 and DG.

We can simultaneously measure CA1 and DG within the same slice, which is not possible with conservative glass electrodes measurements or by low frequency electric stimulation. Low frequency electrical stimulation is not able to induce LTD in adult brain slices of rats and mice, but induce LTD in only developing brain slices. Therefore NMDA-induced chemical LTD method is more useful for the detection of endocrine disrupters than electrical LTD induced by low frequency stimulation.

A receptor responsible for the BPA effect is probably ERRγ, since BPA has a high affinity to ERRγ (nearly 100–1000 fold more affinity than ERα) (Takayanagi *et al.* 2006; Tanabe *et al.* 2012), and the effect of BPA on LTD was suppressed by 4-OH-tamoxifen, an antagonist of ERRγ (Figure 1B). In addition, BPA-induced enhancement of spinogenesis (i.e., increase in the spine density) is suppressed by 4-OH-tamoxifen (Tanabe *et al.* 2012). Although 4-OH-tamoxifen is an antagonist of not only ERRγ but also estrogen receptors (ERα/ERβ),

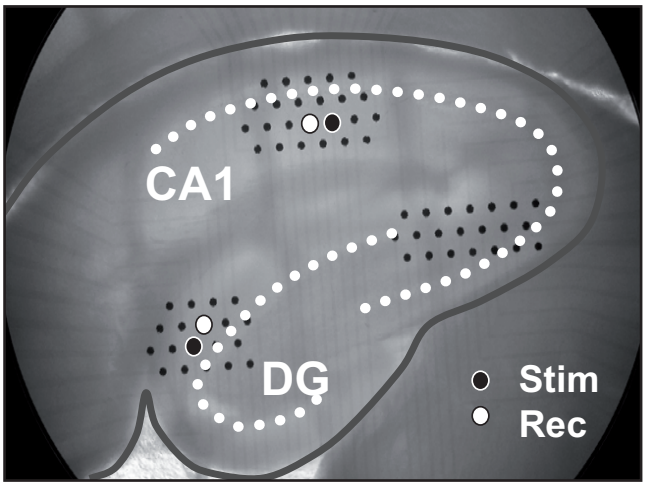

Fig. S1. Custom multi-electrode probe. The arrangement of stimulation electrodes (black circle) and recording electrodes (white circle) on a custom multielectrode probe (dotted line represent pyramidal and granule cell layers).

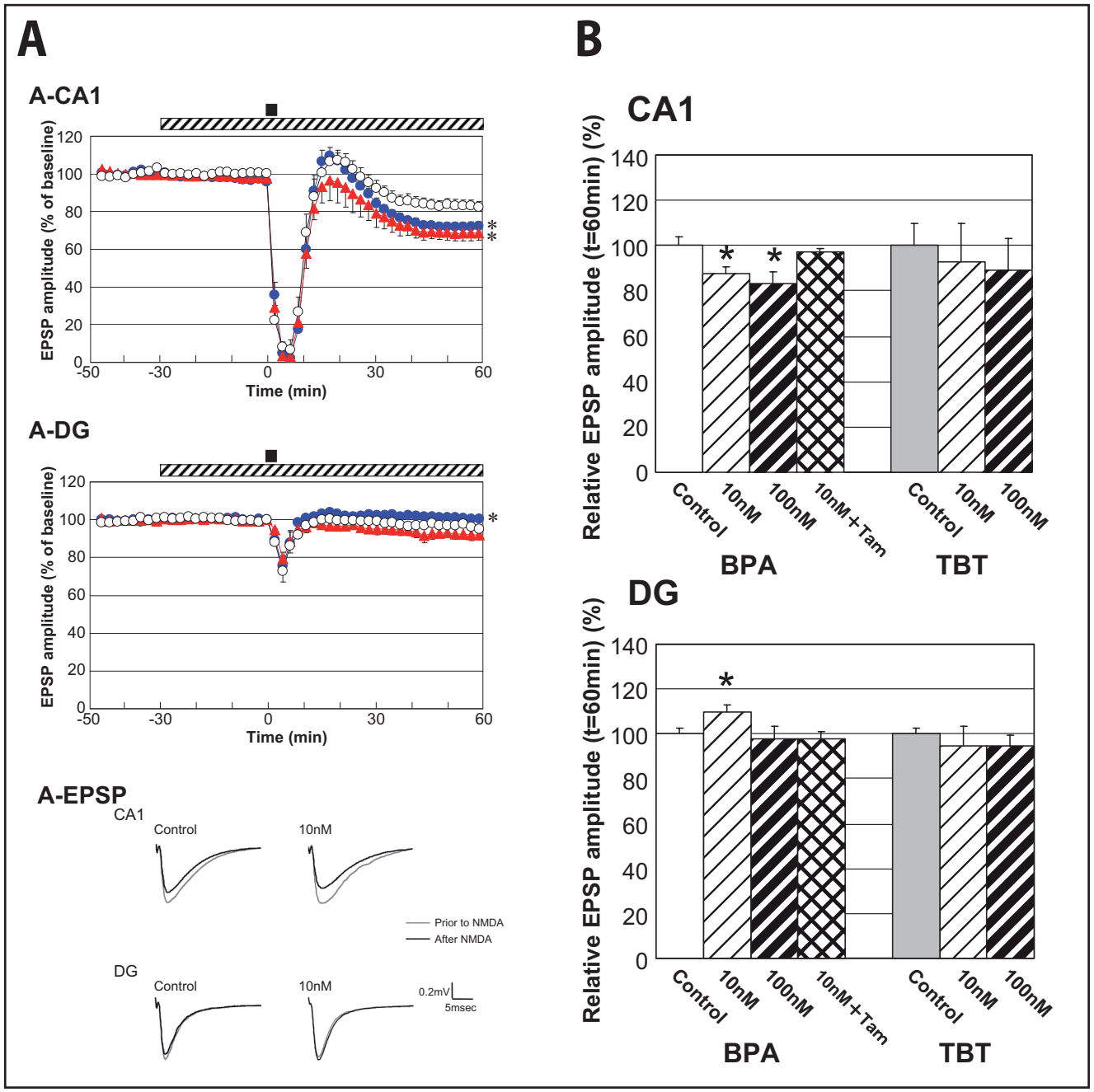

Fig. 1. Effects of BPA and TBT on LTD in adult male rat hippocampus.
(A). Rapid modulation of LTD by BPA in the CA1 (A-CA1) and DG (A-DG) of the same hippocampal slice. BPA concentration was 0 nM (open circle), 10 nM (blue circle), 100 nM (red triangle), respectively. Vertical axis indicates maximal amplitude of EPSP. Here, 100 % refers to the EPSP value at t=–40 min prior to NMDA stimulation, irrespective of the test condition. LTD was induced by 30 μM NMDA perfusion at time, t=0 to 3 min (closed bar above the graph). Hatched bar above the graph indicates period of time during which BPA was administered. (A-EPSP), EPSP traces in the presence of 10 nM BPA, showing sample recordings taken prior to (t=–40 min) and after (t=60 min) NMDA stimulation.
(B). Comparison of modulation effect of LTD by BPA and TBT in the CA1 and DG of hippocampal slices. Vertical axis is relative EPSP amplitude at t=60 min, where EPSP amplitude at t=60 min of the control slice without drug application is taken as 100%. From left to right, BPA and TBT at indicated concentrations. In 10 nM BPA experiments, 4-OH-tamoxifen treatment is also indicated (10 nM + Tam). The significance of the endocrine disrupters' effects were confirmed with 60 min EPSP level via Tukey-Kramer post-hoc multiple comparisons test when one way ANOVA tests yielded $p<0.05$. (*, $p<0.05$). Illustrated data points and error bars represent the mean ± SEM from n=8–12 independent slices from 4–6 rats.

ERα/ERβ are excluded as a receptor for BPA, because BPA-induced modulation of LTD and BPA-induced spinogenesis are not suppressed by ICI 182,780, an antagonist of ERα/ERβ (Tanabe *et al.* 2012).

We could speculate mechanisms of BPA-induced enhancement of CA1 LTD in the following way. Binding of BPA to ERRγ induces MAPK activation, as observed in spinogenesis (Tanabe *et al.* 2012) and then phosphor-

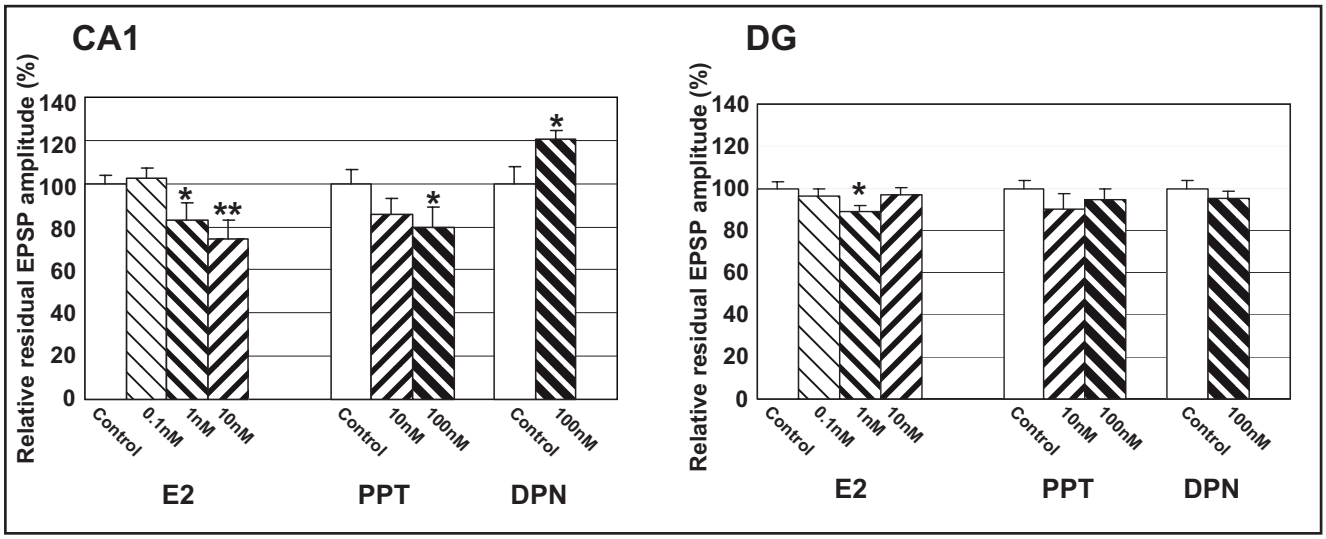

Fig. S2. Comparison of modulation effect of E2, PPT, and DPN on LTD in the CA1 and DG of adult rat hippocampal slices. Data are modified from (Mukai *et al.* 2007). Vertical axis is relative EPSP amplitude at t=60 min, where EPSP amplitude at t = 60 min of the control slice without drug application is taken as 100%.

ylation of MMDA receptor occurs. NMDA perfusion-induced increase in calcineurin activation is enhanced due to this phosphorylation of MMDA receptors, resulting in enhancing dephosphorylation of AMPA receptors. These events lead to the enhancement of LTD. Interestingly in DG region, low dose BPA (10 nM) suppressed LTD, but high dose BPA (100 nM) had no effect on LTD. These results suggest that BPA is an endocrine disruptor, but not a simple xenoestrogen. We should further investigate the DG-mechanisms which might suppress calcineurin or MAPK by ERRγ upon binding of BPA. When the concentration of BPA was above 100 nM, non-specific effect may occur resulting in no effect on LTD. Note that when BPA concentration is high, BPA effect on Ca^{2+} signaling was abolished due to nonspecific effects (Tanabe *et al.* 2006).

In addition, we examined BPA-induced modulation of LTP. The LTP induced by tetanic stimulation in the hippocampal CA1 region showed approximately 134% of EPSP slope at t=60 min, in comparison with control EPSP slope is set as 100%. Perfusion of BPA from t=–30 min to t=60 min did not significantly change the slope of LTP. BPA was not able to further enhance LTP which was established by strong tetanic stimulation. A similar no enhancing effect on tetanic stimulation-induced LTP was also observed by 1 nM estradiol perfusion (Ooishi *et al.* 2012).

BPA effects on LTD or LTP might depend on specific regions of the brain. In striatum, BPA-induced modulation of dopamine receptors is observed for LTP and LTD (Zhou *et al.* 2009). Prenatal and neonatal exposure to BPA suppressed LTP (incused by theta burst stimulation) at postnatal day 10–15, and suppressed LTD (incused by theta burst stimulation) at postnatal day 20–35.

One of a high affinity binding protein of TBT is retinoid X receptor (RXR) (Nakanishi *et al.* 2005). In the current study, TBT did not show any effect on LTD, suggesting that RXR is not involved in the rapid modulation of LTD.

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Disclosure statement:
The authors have nothing to disclose.

Conflict of Interest:
The authors declare no competing financial interests.

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