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Bisphenol-A antagonizes the rapidly modulating effect of DHT on spinogenesis and long-term potentiation of hippocampal neurons

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HIGHLIGHTS

- BPA rapidly increased the densities of dendrite spine and synapse in hippocampal neurons.
- ERs antagonist but not ARs antagonist blocked BPA-enhanced densities of spine and synapse.
- Inhibitor of ERKs or p38 eliminated BPA-increased densities of spine and synapse.
- BPA eliminated the effects of DHT or 17β-E_2 on densities of spine and synapse and LTP.
- BPA enhanced LTP, which was blocked by p38 inhibitor but not by ARs antagonist.

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ABSTRACT

Bisphenol A (BPA), a common environmental endocrine disruptor, modulates estrogenic, antiestrogenic, and antiandrogenic effects throughout the lifespan. Recent studies found more obvious adverse effect of BPA on some neurobehavior in males than that in females. In this study, BPA at 10–100 nM rapidly increased the densities of the dendrite spine and synapse in cultured hippocampal neurons of rats *in vitro* within 1 h. Co-treatment of BPA (100 nM) with dihydrotestosterone (DHT, 10 nM) or with 17β-E_2 (10 nM) completely eliminated the promotion of DHT or 17β-E_2 in the densities of the dendritic spine and synapse. Pretreatment of estrogen receptors (ERs) antagonist ICI182,780 but not of androgen receptors (ARs) antagonist flutamide (Flu) for 30min completely blocked BPA-enhanced densities of the dendritic spine and synapse. Pretreatment of flutamide for 30min before BPA and DHT completely rescued BPA-enhanced densities of the dendritic spine and synapse. Furthermore, pretreatment of ERK1/2 inhibitor U0126 or p38 inhibitor SB203580 entirely eliminated BPA-induced increases in the densities of the dendritic spine and synapse. Meanwhile, BPA (100 nM) enhanced long-term potentiation (LTP) induction of dentate gyrus in hippocampal slices of younger male rats, which was not blocked by co-incubation of flutamide but was inhibited by pretreatment of an P38 inhibitor SB203580. Co-application of BPA with DHT inhibited DHT-suppressed LTP. These results are the first demonstrating the antagonism of BPA to the rapid modification of DHT in synaptic plasticity. However, BPA alone rapidly promotes spinogenesis and synaptic activity through ER instead of AR, and both ERKs and p38 signaling pathways are involved in these processes.

1. Introduction

Bisphenol A (BPA), one of the most representative phenolic compound in environmental endocrine disruptors, is widely used in the manufacture of epoxy resins, polycarbonate plastics,

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Abbreviations

ACSF	artificial cerebral spinal fluid
3α-diol	5α-androstane,17β-diol-3α-diol
3β-diol	5α-androstane,17β-diol-3β-diol
17β-E_2	17beta-estradiol
BPA	bisphenol A
CREB	cAMP-response element binding protein
CNS	central nervous system
DG	dentate gyrus
DHT	dihydrotestosterone
DIV	day cultured *in vitro*
DMEM	Dulbecco' Modified Eagle Medium
EED	environmental endocrine disruptor
ER	estrogen receptor
ERK	extracellular signal-regulated kinase
FBS	fetal bovine serum
F-actin	filamentous actin
fEPSP	field excitatory postsynaptic potentiation
Flu	flutamide
GDX	gonadectomied
HFS	high frequency stimulation
ICI	ICI182,780
LCSM	laser confocal scaning microscope
LIMK	LIM kinases
LTD	long-term depression
LTP	long-term potentiation
MAPK	mitogen-activated protein kinase
mPFC	medial prefrontal cortex
Ovx	ovariectomy
p-ERK	phosphorylation of extracellular signal-regulated kinase
PKA	protein kinase A
SB	SB203580
S.E.M	standard error of mean
Syn I	synapsin I
T	testosterone
TP	testosterone propionate

bactericide, antioxidant, liners for food and beverage cans (Iwakura et al., 2010; Kim et al., 2007; Asimakopoulos et al., 2012). Given its similar chemical structure to diethylstilbestrol which has high affinity to estrogen receptor (ER), a number of earlier studies focused on the estrogenic properties of BPA (Rathee et al., 2012). Our previous studies found that BPA inhibited 17β-E_2-induced increases in the densities of dendritic filopodium and synapse of hippocampal neurons *in vitro* (Xu et al., 2010, 2014). Recent study of our laboratory indicated that co-administration of 17β-E_2 with BPA (100 nM) abolished 17β-E_2-induced enhancement of baseline field excitatory postsynaptic potentiation (fEPSP) and long-term potentiation (LTP) suggesting a anti-estrogenicity of BPA (Chen et al., 2017). However, increasing findings showed a sex-specific influence of BPA on the development of the brain and behavior with more obvious adverse effect of BPA on some neurobehavior in males than that in females (Fujimoto et al., 2013; Xu et al., 2011b, 2013, 2015a). BPA impaired visual and spatial memory of adult male rats in object recognition and object placement tasks (Eilam-Stock et al., 2012) and spatial memory of young male mice (Kim et al., 2011). Our previous studies showed that exposure to BPA in both adolescent and adulthood impaired spatial memory and avoidance memory in male mice but not in female mice (Xu et al., 2011b, 2013). Recently, using gonadectomied (GDX) male mice, we further found that long-term exposure to BPA did not change the spatial memory of GDX mice but inhibited testosterone propionate (TP)-rescue of impaired spatial memory in GDX mice (Fang et al., 2017). Thus, we hypothesis that the effect of BPA exposure on male's behavior may be refer to interfere the modulation of androgen in brain.

Hippocampal synaptic plasticity, closely associated with memory and emotion, is modulated by endogenous androgen in males. Gonadectomy decreases spine-synapses in male rat hippocampus, and the supplement of dihydrotestosterone (DHT) or testosterone (T) for 3 days rescues the spine—synapse density of CA1 neurons in GDX rats (Leranth et al., 2003). Synaptic LTP are usually induced in the hippocampus by high frequency stimulation (HFS) and are regarded as the cellular substrate for learning and memory (Mauro et al., 2015). Electrophysiological study showed that T rescues EPSP in hippocampal slices decreased by castration in rats (Smith et al., 2002). For rats castrated during puberty, T and DHT decreased hippocampal LTP *in vivo* (Harley et al., 2000), and flutamide, an antagonist of androgen receptor (AR), suppresses this reduction (Hebbard et al., 2003). It was found that BPA for 4 d prevented T-induced spine synapse formation in the hippocampal CA1 and the medial prefrontal cortex (PFC) regions of adult GDX or gonadally intact male rats (Leranth et al., 2008). Early exposure to BPA alters the number of neuron and glia in the rat PFC of adult males but not females (Sadowski et al., 2014). Adolescent BPA exposure for one week decreases the dendritic spine density on medial PFC basal dendrites more pronounced in males than that in females (Bowman et al., 2014). Our recent study found that long-term exposure to BPA decreased the synaptic density and had a negative effect on remodeling the synaptic interface of the hippocampus in both intact and TP-treated-GDX mice (Xu et al., 2013; Fang et al., 2017).

In addition to slow effects, androgenic steroids in the brain rapidly modulate synaptic plasticity of hippocampus interacting with specific membrane receptors. Hatanaka and co-workers found that the application of 10 nM DHT or T rapid increased the density of spines of CA1 pyramidal neurons within 2 h in hippocampal slices from adult male rats; however, the rapidly enhanced spinogenesis was blocked by hydroxyflutamide, a specific inhibitor of AR (Hatanaka et al., 2009, 2015). In the CA1 region of hippocampal slices of male rat, induction of long-term depression (LTD) and depotentiation by low frequency stimulation (15 min, 1 Hz) was fully prevented under finasteride, an inhibitor of DHT synthesis, but rescued by exogenous DHT (Mauro et al., 2015). DHT was also involved in a small LTP induced by weak frequency stimulation (1 s, 25 Hz) in the CA1 hippocampal slices of male rat (Mauro et al., 2015).

Recent experiments revealed that acute BPA exposure to adult male rats significantly decreased dendritic spine density on pyramidal cells in CA1 and medial PFC *in vivo* (Eilam-Stock et al., 2012). An AR-mediated reporter gene assay verified the antiandrogenic activity of BPA (Sun et al., 2006; Xu et al., 2005). Because TP in the brain can be partially aromatized into estradiol (E_2) which is well-established regulators of spinogenesis and synaptic plasticity of both sexes, in the present study, using a non-aromatizable androgen, dihydrotestosterone (DHT), we investigated the anti-androgenic effect of BPA on spinogenesis and synaptic plasticity of hippocampal cells cultures and hippocampal slices *in vitro* and underlying mechanisms.