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# NON-CLASSICAL BIOACTIVITY OF ENVIRONMENTAL ESTROGEN BISPHENOL A, WITH A FOCUS ON NEUROPHYSIOLOGY

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**ABSTRACT**
Bisphenol A is synthetic biphenolic intermediate used in making polycarbonate plastic, epoxy resin and flame retardants. It's the most ubiquitous environmental estrogen and endocrine disruptor, to which human population is exposed across the globe. Classical genomic effects are toxic to developmental and reproductive biology. Feminizing effects have threatened existence of some aquatic species and seriously depress human sperm count. The complex multifaceted mechanisms of diverse bioactivity of bisphenol A need urgent understanding, given the magnitude of hazard. Activity Spectrum of bisphenol beyond classical genomic effects, currently leads to new revelations of steroid hormone physiology. Very importantly the effects on nervous function have attracted attention. The progress in this specific context is obviously, crucial to evolving appropriate preventive and corrective strategies for exposed massive population. The aspect is briefly reviewed.

**KEYWORDS:** Bisphenol A, Endocrine disruptor, xenoestrogen, environmental toxicology, biphenolics.

## BASIC PROPERTIES AND THE ENVIRONMENTAL CONTEXT

Bisphenol A (BPA) is synthetic molecule comprising two phenol rings in structure, connected by a methyl bridge, with two methyl groups attached to the bridge. Molecular weight is 228gm/mole, empirical formula $(CH_3)_2C(C_6H_4OH)_2$, specific gravity 1.09-1.19 gm/cm3, and solubility 120-300 mg/L, at pH 7.0. Bio-degradation in atmosphere is 76-95% in 28 days.

It was synthesized in 1891 and its estrogenicity was first reported in 1936 (Dodds and Larsson 1936). Use of BPA in plastics opened its massive use and public health concerns over estrogenicity. It is among the highest volume compounds in world with annual production over 6 billion pounds and release in atmosphere over 100 tons (Vandenberg et al 2009). Domestically produced BPA is used as intermediate in production of polycarbonate plastic and epoxy resins, flame retardants and other specialty products. Polycarbonate plastic is used to make variety of common products including baby and water bottles, sports equipments, medical devices etc. Epoxy resins are used as coatings to line inside of almost all food and beverage cans to avoid metal contact of the contents. From the food cans alone, on an average 6.6 micro gm/daily/person BPA exposure is likely. Warmed plastics leach out more BPA. Effluents from waste water treatment plants or raw sewage water are major environmental reservoirs of BPA, to contaminate aquatic environment.BPA in surface river water can be adsorbed to sediments. In anaerobic or semi-aerobic sediment environment, BPA can persist for long periods. Under aerobic environment it is degraded more than 90%.Environmental BPA concentrations even below 5micro gm/L induce adverse effects in multiple vertebrate and invertebrate beings, mainly causing reproductive and developmental effects). Endocrine disruption refers to endocrine effects similar to or opposite to hormones but in total irrelevance and disjunct with physiology. They cause adverse effects in humans and wild life subsequent to changes in endocrine function. Low doses of EDC can cause undesirable effects if exposure is continuous (Sumpter & Johnsson 2005).

BPA is massively used, although moderately estrogenic.(four orders of magnitude, below 17beta estradiol). The estrogenic activity of endocrine disruptor chemicals (EDC) is presented as 17beta estradiol equivalent (E2eq), according to their relative estrogenic potency. Potency of estradiol is 1. Investigated river water samples show estrogenicity in range of 0.02 to 2.99 ng E2eq/L, with mean 1.16 ng E2eq/L. Surface water concentration of BPA in river was found in range of 1.5-8.2 ng/L, median 3ng/L. (Gong et al 2009). Environmentally prevalent BPA concentration affects medaka, brown trout, zebra fish, mollusks and copepods. The sediment dwelling (Benthic) organisms are likely to receive much higher exposures as BPA concentrations are higher in sediment than water column.

**RECEPTOR ACTIVITIES OF BPA**

Endogenous estrogens are physiologically important signaling molecules that can vary in concentration by an order of magnitude within a reproductive female. Foreign estrogens are also found in blood stream (Grace et.al, 2004) with scope of altering estrogen signaling in variety of cell types (Gertz et.al, 2012). US-EPA defines environmental endocrine disruptor or Endocrine Disruptor Chemical EDC, as "an exogenous agent that interferes with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental process (Kavlock et.al, 1996).

Action of BPA at estrogen receptor types, ERalpha (ERα), ERbeta (ERβ), ERR (estrogen related receptor) and mER (estrogen membrane receptor) have been documented. These receptors are part of large nuclear steroid hormone receptor super family with some cross-reactivity of the ligands. For example, both estrogen and BPA bind to the thyroid receptor and antagonize androgen receptors. Invertebrates have variant ER, which does not bind estrogen, similarly ERs do not bind estrogen. BPA is considered weak estrogen based on the 1000 to 10000 time lower affinity for binding the (ERα) and ERβ, as compared to E2 (Alonso-Magdalena et.al, 2012). This gives Effective Concentration in 50% of tests (EC50) as under. BPA EC50= 2-7x$10^{-7}$ M; Es EC50 = 1-6x$10^{-13}$ M (Fang et.al, 2000). BPA has higher affinity for ERβ than for ERα (Kuiper et.al, 1997; Routledge et.al, 2000). BPA acts as estrogen agonist via ERβ and exhibits mixed agonist antagonist activity through ERα (Kurosawa et.al, 2002). BPA low dose is physiologically relevant dose (for in vivo studies levels below the current lowest observed effect level (LOAEL) of 50 µg/kg/day were considered as low dose (Wethorill et.al, 2007). For in vitro studies, equivalents of circulating levels at LOAEL of less than 50 ng/ml or less than 2.19x$10^{-7}$ M (Welshon et.al, 2006).

Plasma membrane associated receptors initiate rapid signaling cascades (Watson et.al, 2010). ERs are such receptors. There are major sex based differences in diseases in which neurotransmitters and their transporters and receptors play a role. Ligands first encountered at cells surface, generally initiate responses to changing environment. Both the transporters and receptors of neurotransmitters can be found in the same specialized membrane compartment as that of the estrogen receptors. These compartments are the cholesterol rich caveolae. Many kinases and phosphatases also reside here. Extra-caveolar group of these enzymes also exist. ER induced kinase and phosphatases effects on neurotransmitter transporters and receptors may be direct or via enzymes in signaling cascade. Acute and selective responses via kinases and phosphatases regulate the transporters of neurotransmitters. Estrogen can activate the kinases and phosphatases. BPA is now known to adversely affect some socio-sexual behaviours, locomotion, spatial learning memory and fear anxiety at relatively low doses. ERα is more important in estrogenic neuroprotection (Yang et.al, 2010). Genetic knock down of ERα but not ERβ in the hippocampus CA1 region, resulted significantly in attenuation of rapid E2 signaling and neurotransmitter effect following global cerebral ischemia. In addition to extra-nuclear ERα, extra-nuclear GPR30 may also participate in mediating the estrogenic rapid signaling and neuroprotection following global ischemia.

BPA appears more potent than E2 on non classical membrane receptors, because **i)** BPA elicits rapid responses via non-classical estrogen triggered pathways; **ii)** BPA may bind differently in ER (Gould et.al, 1998); **iii)** Co-activator recruitment: BPA + ERβ complex has 500 fold greater potency than BPA/ERα in recruiting co-activator TIF2. This is reflection of more efficient capacity that ERβ has to potentiate receptor gene activity in some cell types (Routledger et.al, 2000). BPA can activate transduction signaling pathways, which can vary from cell types to cell types. Total disruptive effect arises from combination of rapid mechanisms and longer signaling effect. Extra-nuclear ERα however mediates rapid BPA effects at low doses. E2 and BPA are equally potent at 1nM dose, produced rapid regulation of $Ca^{2+}$ signals in pancreatic beta cells (Nadal et.al, 2011). A molecular pathway different from ERα and ERβ was suggested.

Background Paper on Bisphenol A and other biochemical/molecular interactions (Thayer KA, Belcher S WHO/HSE/FOS/11.1 Geneva WHO 2011) summarizes following:

BPA can interact with many other receptors, as seen in vitro. It is anti-androgen (IC50 0.8 to 19.6 µM/L). In another study, androgen receptor antagonism by BPA showed IC50 2.34 µM/L. This is 10 fold higher than median effective concentration EC50 for ERα agonist activity, i.e.0.272 µM/L.

BPA can interact with non-classical ER system at similar or lower conc. BPA has high affinity for ERR-gamma (which does not bind E2). IC50 value of BPA for ERR-gamma is 13.1nM/L. This is 80 to 100 times lower than IC50 value of BPA for ERα (1040 nM/L) or for ERβ (IC50=1320 nM/L). The ERR can bind functional estrogen response element and the ERR response element. The ERα and ERR-gamma systems may crosstalk to mediate BPA activities. Human ERR-gamma is preferentially bound by BPA with 105 times greater affinity than ERα (Okada et.al, 2008). ERR family includes ERRα, ERRβ and ERR-gamma. ERR-gamma is expressed strongly during development in brain and subsequently in other tissues also. Highest ERR-gamma expression is found in placenta. The physiological roles remain unclear.

BPA binds both thyroid TRα and TRβ receptors with relatively low affinity. At physiological BPA concentration (below 0.1 µM/L), no thyroid effects occur. BPA is also agonist of glucocrticoid receptor. BPA affects receptors linked to neural activities at higher concentrations. Effective BPA conc. range from 10.8 µM/L for $5HT_6$ receptor to 27-38 µM/L for $DA_2$ receptor, opioid-mu1 receptor and opioid receptor like receptor.1. Lowest observed effect concentration (LOEC) of BPA for nervous tissues ranges from 0.0000001 to 2.5 µM/L. Agonist activities of BPA on ERα (ESR1) is significant factor in relative ranking of endocrine activity of agents. AC50 or lowest effect concentration (LEC) values of BPA from the four ESR1 assays included in Tox21 ranges from 0.64 to 1.72 µM/L.

Biochemical and molecular interactions of BPA appears complex. These involve classic ERs and also a variety of other receptor systems and molecular targets. It is unclear if all observed in vitro effects can occur in vivo at concentration relevant to human exposure, and whether observed changes would lead to adverse health outcomes. Rapid BPA signaling effects involve proteins similar as nuclear hormone receptors ERs. Some receptors/signaling pathways involve alternative transmembrane G protein coupled receptor GPR30. At GPR30 an IC50 of BPA was found to be 630 nM and relative binding capacity, 2.83 nM, compared to 17beta E2 (Thomas and Dong, 2006). The RBA of BPA for binding GPR30 is higher than that observed for ERα and ERβ. BPA is reported to bind G protein coupled receptor or GPER (earlier called GPR30), with IC50 value 0.630 µM/L which is 2 to 3% of IC50 value for Estradiol (0.0178 µM/L). Both estradiol and BPA 200 nM caused increase in cAMP mediated through GPR30. In HEK cell lines that bear no ERs.

## BPA EXPOSURE AND LOAD

The EPA (environment protection agency, USA) declared 50 µg/kg/day as safe limit for BPA intake (USA, EPA, 2010). LOEL for BPA is 50 mg/kg/day. Human urinary concentrations range between 0.024 to 8.5µM. BPA concentration in human serum ranges from 0.2 to 1.6 ng/ml (0.88-7 nM). The higher range of human serum BPA levels is 0.001 to 0.3 µM in high risk population chronically exposed to high levels of BPA, eg industry workers. Similarly individuals with limited metabolic capacity eg, prenatal and neonatal cases, can have such conc.

Following administration of 5 mg of BPA, the solely detected metabolite in blood and urine was d(16) BPA glucuronide. Its concentration in blood and urine were below detection levels respectively 10 nM and 6 nM. Glucuronide was cleared from blood in to urine with terminal half life less than 6 hour. Whole dose was recovered in urine as glucuronide. Highest blood level of the glucuronide was measured 80 minutes after oral administration (Volkel et.al, 2002). Humans rapidly clear BPA as there is no entero-hepatic circulation of BPA, like the rats have. BPA 10 mg/kg intra venous or 10 to 100 mg/kg oral were administered to groups of female rats. Blood samples were examined by GCMS. Detection was sensitive up to 10 ng/ml plasma BPA. Immediately after I.V. injection, plasma concentration was 15 µg/ml. After first hour it declined rapidly to 700 ng/ml, further to 100 ng/ml by 2 hours. No detection was possible at 24 hours. On oral administration of 10 mg/kg in rats plasma BPA was detectable as early as 10 minutes. Maximal plasma level reached one and half hour later (31 ng/L) and at 6 hour, 40 ng/ml. Animals given 100 mg/kg dose showed within 30 minutes, plasma levels 150 ng/ml, at 3 hours the conc. was 134 ng/ml. Undetectable level in 48 hours was reached (Upmeier et.al, 2000). Low bio-availabilities of 16.4% for 10 mg/kg and 5.6% for 100 mg/kg doses were observed.

BPA serum concentration in women is approximately 1-2 ng/ml (about 5-10 nM/L) (Ikezuki et.al, 2002). BPA conc. in amniotic fluid is approx 8.5 ± 0.2 ng/ml (about 40nM/L). In blood can reach in µMolar range in case of high oral or intravenous dose. Additionally BPA may augment effect of natural hormones, in combination.

## THE BIOLOGICAL ACTIVITY PROFILE OF BPA

Besides mimicking estrogen at nuclear receptor level, disruptive actions of BPA include effect on androgen system, thyroid function, and developmental differentiation and function of CNS, and on immune system. BPA can directly impair intracellular transduction pathways, independent of nuclear action. BPA metabolic and pharmacokinetic effects influence bioavailability of steroid hormones. Such secondary effects include modification of $Cytochrome_{P450}$ metabolic enzyme expression and activity. Serum hormone binding protein expression and interaction may also be altered. BPA has potential to transmit effects of early life exposure to physiological expression later in life and across generation via epigenetic mechanisms.

### Nervous System Effects

Of more than a lakh commercial chemicals only some10% (excluding drugs), have been examined for neurotoxicity (Landrigan et.al, 1993). Environmental neurotoxicants may produce wide range of even subclinical effects. These may include, reduction in intelligence, impairment in reasoning ability, shortening of attention plan and alternation of behavior. Environmental endocrine disruption is recognized as health issue since 1992 (Colborn et.al, 1993). Endocrine disruptors may influence proteins and enzymes relating neurotransmitter system, via genomic effects (Ponzica et.al, 2011). Human health effects of BPA exposure are predicted from studies in mice and rat mostly. The low dose BPA and its effects refer to less than 5 mg/kg/day and are different from higher dose effects. Both direct and indirect BPA effects on nervous system are known.BPA can affect brain development through disruption of sex hormone function. Disruption of

thyroid hormone function also must affect nervous system development.

Bisphenol exposure can be verified in urine samples of around 93% human population in US. Findings in animal models of BPA exposure include masculinization of brain anatomy in females, impaired maternal behavior, reduced aromatase activity, altered dopaminergic function, impaired learning, increased aggressive behavior, altered infant behavior in male monkeys toward mother, and altered attractiveness of males to females. BPA is a risk factor for other kinds of health endpoints as well as neurotoxicity. These include immune system function, distorted memory development, sperm abnormalities, prostate cancer cardiovascular disease and obesogenicity (Weiss, 2012).

Neurogenesis, the characteristic of early development, can be influenced by hormonal imbalances. Adult neurogenesis is different, but may emulate developmental processes. More than 20% of men and 33% of women above age of 65 years may be destined to acquire dementia over lifetime. In next 50 years the dementia prevalence may get four fold with increasing longevity (Taupin, 2006). Estrogen like, neuron proliferation and enzyme induction is caused by BPA, even without ER binding (Welshom et.al, 2006). BPA is not conventional estrogen, and also exhibits antagonistic effect in some respects (MacLusky et.al, 2005a). Neuronal Long Term Potentiation induced by estradiol was inhibited by BPA co-administration (Mukai et.al, 2006).

Estrogens are neuroprotective. Absence of estrogen during prenatal life in Turner syndrome is associated with cognitive dysfunction and psychosis. Directly, BPA can cause neuro-degeneration via generating oxidative stress. The dysfunction of DA system associates with neuropsychiatric disorders eg. Parkinsons disease, schizophrenia, attention deficit hyperactivity disease (ADHD), and autism. Significant BPA effect on functioning of brain dopaminergic (DA) system and hippocampus, which serves cognition functions, are demonstrated. Sex steroid hormones appear associated with development of brain DA system. Prenatal and neonatal BPA exposure alters D1 dopamine receptor expression and density in male mice. BPA affects development of DA pathways in a manner linked to gender (Suzuki et.al, 2003). Prenatal BPA exposure blunts dopaminergic reward system only in female and not male off-springs (Laviala et.al, 2005). BPA role in producing ADHD appears to be through induced hyper-functioning of DA system (Ishido et.al, 2007). BPA exposure of neonatal rats led to significant hyper activity at 4-5 weeks of age. By $8^{th}$ week there was significant decreased expression of DA transporter gene (Mizuo et.al, 2004; Ishido et.al, 2005). These findings support BPA induced DA hyper-function and ADHD.

Declarative memory represents the ability to form memory of every day facts, and events through personal experience and construction of reality within the consciousness. Hippocampus plays important role in declarative memory. The sex hormones programme the hippocampus differently in the two sexes (Maclusky et.al, 2005b). Thyroid hormones also play role in hippocampus development and maturation. Since BPA disrupts estrogen and thyroid functions, it can potentially impact hippocampal growth and function (ishido and Masuo, 2014; Elsworth et.al, 2013). Memory loss caused by BPA is demonstrated in "step-through" passive avoidance behavioural test. BPA exposure in neonatal mice and testing of spatial learning capacity on Morris water maze was performed between 34-37 postnatal days. Generally, the male animals learn better on this test. Gender dependant pattern of learning acquisition was abolished in low dose BPA exposed animals. It is implied that BPA exposure may interfere with development and expression of normal sex differences in cognitive function, via inhibition of estrogen dependant hippocampal synapse formations (Carr et.al, 2003).

BPA effects were investigated for rapid modulation of synaptic plasticity in hippocampus of adult male rats (Inagaki et.al, 2012; Xu et.al, 2014; Liu et.al, 2015). Both in the CA1 and CA3 hippocampal pyramidal cells, BPA enhanced LTD significantly. The LTD in dentate-gyrus of hippocampus was suppressed (Ogiue-Ikeda et.al, 2008). Both long term potentiation (LTP) and long term depression (LTD) are essential in processing of memory. LTD is probably, a mechanism to rectify wrong memories formed vide LTP.

BPA alone does not affect long term potentiation induction. When BPA is co-perfused with estradiol, there is complete suppression of LTP enhancing effect of estradiol. BPA given from embryonic stage to 3 postnatal weeks in rat pups increased hippocampal local estrogen production. The target of BPA action appeared to be ERα and steroid-genic enzymes (Kawato, 2004).

Modulation of human recombinant nicotinic receptors by estrogens and xenoestrogens was demonstrated (Nakazawa and Ohno, 2001). For genomic pathway of steroid action at least 30 minute delay is mandatory (Orimo et.al, 1993). BPA inhibited cloned nicotinic receptor more strongly than 17β E2. Tamoxifen also inhibited. As estrogens and BPA are both hydrophobic, they may not directly bind the receptor but exert effect through the membrane. However, the inhibitory potency appeared to correspond with hydrophillicity (Garbus et.al, 2001). The observations may be clinically important, since nicotinic receptors are linked to epilepsy, schizophrenia etc (Gotti et.al, 1997). Estrogen modulates brain NMDA receptors. Role of ER subtypes is not defined. NMDA receptor binding in CA1 pyramidal hypothalamic neurons decreases by ovariectomy. ERα agonist PPT prevents such decline. ERβ agonist DPN has no such effect (Morissette et.al,

2008). Some biological effects of endocrine disruptors are mediated by aryl hydrocarbon receptor (AhR), the key players in cellular defence against various xenobiotic. AhR-ER pathways interplay at several levels (Swedenborg and Pongratz, 2010).

**Cardiovascular Effects**

Epidemiologic studies suggest relation of BPA exposure to cardiovascular disease, eg. atherosclerosis, coronary artery disease and heart rate variability deficits. Decreased heart rate variability by BPA exposure may indicate alteration in ion channel currents during pacemaker depolarization. BPA exposure levels depend on lifestyle factors. Neonates in ICU, show urinary excretion of 4.4 nM to 4 µM, while industrial workers 0.024 to 8.5 µM. Human serum BPA ranges from 0.001 to 0.3 µM in adults. The physiological BPA concentration in serum of women is however 0.64 ng/ml (2.8 nM/L) and in male 1.5 ng/ml (6.5 nM/L) (Takeuchi and Tsutsumi, 2002). BPA 10 nM treatment daily for 3 days of cultured bovine adrenal medulla cells, caused stimulation of catecholamine synthesis (Yanagihara et.al, 2004).

Effect of hormonal status on electrophysiology of mouse heart in close-chest in vivo model was studied (Saba et.al, 2002). Ovariectomised mouse show shortened PR interval and ERP of Right ventricle. Estrogen replacement normalizes both. Estrogen thus, prolongs AV nodal conduction and right ventricular effective refractory period. Molecular pathways however need to be elaborated. Posnack et.al, (2014), studied BPA effect on electrical conduction in isolated heart. Changes in cardiac conduction of whole heart from female rats appeared at 0.1 µM. Changes in cardiac conduction occurred with BPA exposure acutely at less than 15 minutes (much less than reported half life of BPA). Small change in ion channel expression and or conduction may significantly cause pathology. The results indicate a prolonged inactivation of sodium channels, as a result of reduced potassium current and longer action potential duration, as effect of BPA. Cardiac effects are pronounced at higher BPA conc. BPA 100 µM caused complete AV block and QRS widening. A reduced conduction through the gap junctions in ventricular muscles may also be contributing to this.

BPA can directly bind to and block Nav 1.5 sodium channel (Oreilly et.al, 2012). These channels bring about phase 0 of depolarization of ventricle muscle. BPA activation of Maxi-K+ channels in coronary smooth muscle is also known (Asano et.al, 2012). Similar interaction with sarcolemma K+ channels may hyperpolarize cardio-myocytes and decrease cardiac excitability.

ER agonists can inhibit voltage gated sodium current and decrease potassium current, also the L-type calcium current. L-type $Ca^{2+}$ current is depolarizing current in SA and AV node and AV block may result. BPA acts possibly through ERs or activation of NO/cGMP pathway. Increased NO levels also can attenuate L-type calcium current. Rapid non nuclear estrogen receptor signaling in CVS is studied in the endothelial cells, in which rapid activation of eNOS, production of NO and vasodilatation. Results (Mendelsohn and Karas, 2010). Estrogen induces eNOS through pathway involving activation of low molecular weight G protein Gxi, the tyrosine kinase src, and serine/threonine kinase Akt and MAPK.

**BPA EXPOSURE AND SIGNALING FUNCTIONS**

**GABAergic function**

Using a conventional whole cell patch clamp technique from acutely isolated rat CA3 pyramidal neurons, BPA effects on GABA currents was studied (Choi et.al, 2007). BPA elicited membrane current in dose dependant manner that could be blocked by bicuculline, the selective $GABA_A$ receptor antagonist. BPA potentiated in concentration dependant manner, current induced by low (less than 10 µM) GABA concentration. Allosteric $GABA_A$ receptor modulating agent's diazepam or ethanol significantly reduced the BPA induced potentiation.

High (more than 30 µM) GABA concentration induced current peak were inhibited by BPA, and desensitization of i(GABA) was accelerated. Steady state i(GABA) following higher conc, were also greatly and non-competitively inhibited by BPA. BPA caused concentration dependant inhibition of amplitude of GABAergic miniature inhibitory postsynaptic currents. This suggests effect of BPA on synaptic $GABA_A$ receptors. The $GABA_A$ receptor modulatory effects of BPA suggest CNS toxic potential of BPA.

**Neuronal Sodium Currents**

Conventional whole cell patch clamp technique study from acutely isolated mouse dorsal root ganglion neurons is employed to study effect of BPA on TTX-sensitive (TTX-S) and TTX resistant (TTX-R), $Na^+$ currents. Extra-cellularly applied 17β estradiol inhibited both TTX-S and TTX-R sodium currents, rapidly, reversibly and in concentration dependant manner. No effect of 17β estradiol was seen on activation curve of $Na^+$ channel. Steady state inactivation curve for TTX-S and TTX-R sodium channels was shifted in hyperpolarizing direction. Estradiol inhibits voltage gated $Na^+$ channels in mouse DRG neurons, through a membrane ER activated PKC-PKA signaling pathway. Estradiol can affect neuronal excitability via modulation of voltage gated sodium currents. A membrane non-permeable estrogen E2-BSA, was equally effective as 17β estradiol, but no effect was seen of 17-alpha-E2. Blockers of PKC (GO 6983) and PKA (H89) blocked the acute effects of 17β estradiol.

BPA inhibits TTX-S $Na^+$ currents and TTR currents, rapidly, reversibly, and in concentration dependant manner (Wang et al 2011). BPA shifted the curve of

voltage gated activation for TTX-S sodium channel in the hyperpolarizing direction. IT did not so change the curve of TTX-R channel. BPA shifted the steady state inactivation curve for TTX-S sodium channel in the depolarizing direction. IT did not so change the curve of TTX-R channel BPA prolonged the time course of recovery from inactivation for both TTX-s and TTX-R sodium currents. BPA mediated inhibition of sodium currents is blocked by inhibitor agents of protein kinase-C (GO 6983) and protein kinase-A (H 89).

Nadal et.al, (2000) suggested that catecholamine receptors may serve as non-genomic receptors both for estrogen and xenoestrogen. Considering its complex modulatory effects on voltage-gated sodium channels, BPA might have potential toxicological effects on the nervous system and lead to a change in excitability of nociceptive afferent fibers (Wang et.al, 2011). The role of voltage gated sodium channels in the transmission of nociceptive and neuropathic pain messages is well established.

**Neuronal Calcium Signaling**

Cell signaling mechanism fundamentals are reviewed in: Kovacic (2010a; 2010b). Alkyl phenols including BPA potently activate signaling responses eg.$Ca^{2+}$ signaling and ERK (Extracellular signaling regulated kinase). Low level chronic exposure alters cell signaling mechanisms. In rat GH3 pituitory cells, 1µM BPA stimulates prolactin release. As low as 1 fentoM BPA concentration also can cause $Ca^{2+}$ influx from extracellular source via L-type channels within 1 minute of exposure (Wozniak et.al, 2005). BPA at 1nM does not cause rapid increases in ERK phosphorylation in these cells, but that is observed in neurons and immune cells. BPA effects are therefore cell and tissue specific. High 20 µM BPA concentrations induce in concentration dependant manner, increase in intracellular free $Ca^{2+}$ levels (Reistal et.al, 2005).

Number of unresolved questions remains on interaction of environmental toxicants with $Ca^{2+}$ channel function (Atchison, 2003). Neurotoxicants may disturb $Ca^{2+}$ channel function by effects on pathways that modulate channel activity, eg. by altering GTP binding protein function; altering phosphorylation of the channel or intracellular free $Ca^{2+}$ concentration that contribute to inactivation properties of voltage gated $Ca^{2+}$ channels. Beta subunit of $Ca^{2+}$ channels play important role in regulating the expression and localization of $Ca^{2+}$ channels. Environmental toxicants may have chronic effect on these subunits, leading to diminished or aberrant expression and placement of channels in membrane. Studies focusing on possible intracellular action of toxicants on $Ca^{2+}$ channel are needed.

BPA binds and inhibits VGCC, comparable to cations, Cd, Co, Mn etc (Deutschmann et.al, 2013). BPA affects all subtypes of $Ca^{2+}$ channels, L.N.P/Q,R,T types to same extent. BPA binds the channels in their resting state. BPA binding site is located on extracellular part of pore forming subunit of channels BPA is highly lipophillic and can reach a binding site at transmembrane part. Although EPA currently considers 50 µg/kg/day BPA intakes as safe (US-EPA 2010), human exposures may be much higher (Taylor et.al, 2011).Workers in epoxy resin industry can have 1000 times higher exposures. The highest measured levels up to 10 µM (Wang et.al, 2012), is within the $Ca^{2+}$ channel blocking concentration range of BPA. High exposure of BPA has also been reported for premature infants under intensive care (Calafat et.al, 2009).

Estrogen and xenoestrogens exhibit rapid non-genomic effect via specific plasma membrane receptors. These interactions lead to a modulation of gating property of many different ion channels in various types of cells, including $K^+$ channel (White et.al, 1995; Liu et.al, 1998). $Ca^{2+}$ channel (Nakajima et.al, 1995; Ruehlmann et.al, 1998). $Cl^-$ channel (Canonaco et.al, 1993; Zhang et.al, 1994).

Compound action potential in frog sciatic nerve displayed bisphenol A induced decrease in amplitude and repolarization time. It is suggested that bisphenol A induced rise in cytosolic $Ca^{2+}$ that activated potassium efflux (Pandey and Deshpande, 2012). A brominated BPA derivative most used as flame retardant, Tetrabromo-BPA, accumulated particularly in striatum region of brain in mice when fed 100 µg/kg. It may be prudent to replace BPA by derivatives lacking action on VGCC.

Gap junctions are collection of intercellular membrane channels through which a variety of ions and secondary messengers diffuse among neighboring cells in most tissues. The gap junction mediates intercellular communication (GJIC) is considered fundamentally important in maintaining tissue homeostasis (Lee and Rhee, 2007). Less attention is paid to effect on gap junction channel. Mohri and Yoshida, (2005) suggested that BPA disrupts intercellular calcium oscillations in mouse oocytes, by inhibiting gap junction IC. BPA dose dependently inhibited GJIC among epithelium derived cultured cells.

**Nitric Oxide Link in Neuronal BPA Effects**

17β-E2 1nM increases endothelial eNOS expression and decreases nNOS expression in para ventricular nucleus respectively after 8 hours and 24 hours. ERβ agonist genistein 0.1 µM has same effect. Selective ERα agonist, PPT 10 nM and the antagonist MPP 1 µM did not have any impact, thus excluding role of ERα in above action of estradiol (Gingerich and Krukoff, 2005). Noguchi et.al, (2002) studied effect of BPA on nitric oxide (NO) synthesis in the murine endothelial cell line, MSS31. BPA (1-100 µM) increased nitrite/nitrate, a stable metabolites of NO, level in culture. BPA stimulates NO synthesis through a non-genomic ER-mediated mechanism. Short-term effects of BPA on NO synthesis were weak but similar to 17beta-estradiol).

BPA, dose dependently suppressed NO production by LPS stimulated macrophages (Yoshitake et.al, 2008). This effect was blocked by ERα receptor antagonist. LPS-induced NF-kappa B activation was significantly diminished by EDC treatment. 8-nitroguanosine formation increased in LPS-stimulated cells, but this increase was inhibited by the tested EDCs. BPA suppresses NO production and NF-kappa B activation in LPS-stimulated macrophages through ER-dependent pathway.

Nitric oxide (NO) containing neurons are widely distributed in CNS. Expression of neuronal NOS (nNOS) is influenced in female rats by estrogen and in male rats by testosterone. nNOS may colocalizes with gonadal harmone receptors. Mice strain with knocked out ERα has markedly reduced neurons expressing nNOS in specific regions (eg. controlling reproduction and sexual behavior). As NOS expression is controlled by gonadal harmone, it changes with changing hormonal profile through the estrous cycle. Class and species specific differences occur in the NOS system of hypothalamus and limbic structures. The results may not have generalizable significance (Panzica et.al, 2006).

Neuronal excitability is a dynamic, rather than fixed variable. It allows adjustment of postsynaptic sensitivity to afferent activity. Modulation of resting $K^+$ currents, which determine resting Vm (voltage maximum) and input resistance Rn, have profound impact on neuron excitability (Ahren et.al, 2002). The KCNK family of $K^+$ channels plays major role in control of both variables in mammalian cells (Bayliss et.al, 2003). Dynamic modulation of the Kv2.1 channel represents a novel mechanism to regulate neuronal excitability. The high level expression of Kv2.1 on body and proximal dendrite of neurons throughout mammalian brain, combined with the extent of effects of altering phosphorylation state on Kv2.1 localization and function, affords neurons a potent and reversible mechanism to regulate intrinsic excitability. Activity dependant modulation of Kv2.1 with resultant depression of intrinsic excitability may provide homeostasis to neuronal function in the face of physiological and patho-physiological increases in excitatory synaptic stimulation (Misonou et.al, 2005).

Bisphenol A depressed spinal monosynaptic and polysynaptic reflexes through ERα dependant nitric oxide mediated mechanisms (Pandey and Deshpande, 2015). Artinian et.al, (2012) reviewed Nitric oxide as intracellular modulator. NO regulates neuronal excitability. Spontaneous action potentials generating in BS neurons of pond snail, were blocked by inhibiting the activity of intrinsic NOS. The neurons got hyperpolarized. Intrinsically generated NO may act on three types of conductances: i) a persistent sodium current i(NaP); ii) voltage gated $Ca^{2+}$ currents $i(Ca^{2+})$; iii) Small conductance calcium activated potassium SK channels. NOS inhibitors 7-nitroindazole and S-methyl-1-thiocitrulline resulted in a decrease in i(NaP). The hyperpolarizing and inhibitory effect of decreased i(NaP) on spontaneous spikes were also caused by an inhibitor of i(NaP), riluzole. Spontaneous and depolarization induced spiking was blocked also by attenuation of $i(Ca^{2+})$, that resulted by inhibition of NOS, soluble guanylate cyclase (sGC) or Protein Kinase G(PKG). This suggests an intrinsic NO controlled $i(Ca^{2+})$ via sGC/PKG pathway. Apamin, the SK channel inhibitor partly prevented the hyperpolarization induced following NOS inhibition. Intrinsic NO may therefore be down-regulating the SK channels. Neurons utilize their self produced NO as an intrinsic modulator of neuronal excitability. In BS neurons, intrinsic NO production is necessary to maintain spontaneous tonic and evoked spiking activity.

## THE OXIDANT ANTIOXIDANT SYSTEM IMPACT

BPA is seen to cause increased hydroxyl (OH) radical production in rat striatum (Obota-kubota, 2000). BPA also enhances the toxin mediated hydroxyl radical formation that associated induction of acute Parkinson's syndrome model. BPA-induced increase in the intracellular ROS (reactive oxygen species) cause apoptosis of neurons. Such effect of BPA is not through estrogen receptors (Lin et.al, 2006). BPA also antagonizes increase in apoptotic enzyme caspase3 caused by staurosporin. Such an action may interfere with apoptotic removal of old brain tissue to laying newer structure, during brain development (Nigishi et.al, 2003). Cytotoxicity of BPA is mediated by MAPKs and ROS (Lee et.al, 2008). Low under 100 µM doses of BPA increase viability in S.H.E cells. Increased intracellular $Ca^{2+}$ induces generation of ROS (Hajnoczky et.al, 2006; Boldyrer et.al, 2004). 25mg/kg oral BPA daily was administered for 45 days in wistar rats. The brain exhibited reduced content of reduced glutathione (GSH), and significantly elevated levels of malonaldialdehyde (MDA). A simultaneous vitamin C administration (60mg/kg/day) along BPA prevented the oxidative changes (Aydogan et.al, 2008).

Xanthinedehydrogenase (XD)/xanthineoxidase (XO) catalyse conversion of hypoxanthine to xanthine and then to uric acid. The dehydrogenase uses NAD+ as electron acceptor and does not produce ROS. Oxidase uses oxygen as electron acceptor producing hydroxyl and superoxide anions. Most hepatic capillary endothelial enzyme is dehydrogenase type. Endogenous oxidants as copper, zinc, selenium ion, peroxynitrite and disulfides monoxides can convert dehydrogenase to oxidase. BPA upto 100 µM/L did not cause this. BPA is metabolized to BPA quinone by cytochrome P450. BPA-Q is strong oxidant and binds to nucleotides and reduced glutathione. DNA binding of BPA-Q causes hepato-toxicity. BPA-Q was seen to convert dehydrogenase enzyme to oxidase type (Sakuma et.al, 2010).

**Effects on Mitochondrial Function**

The presence of ERs on brain mitochondria was demonstrated. Their presence, function and identities of protein isoforms are unsettled (Yang et.al, 2004; Strone et.al, 2005). BPA and some analogues reduce mitochondrial function.BPA is partitioned preferentially in membrane containing interior hydrophobic protein (rather than polar part). Mitochondrial membrane has such composition. So BPA accumulates in mitochondrial membrane and causes uncoupling of oxidative phosphorylation, thereby inhibiting Complex1, activity (Nunez et.al, 2001). BPA degrades complex1. In, GC1 cells production of reactive oxygen species upon BPA administration, peaked at 12 hours, and then declined at 24 and 48 hours. In case of the Neuro2A cells ROS production continued (Ooe et.al, 2005). BPA accumulates in tissue, increasing risk of Parkinsonism. Additionally, smoking, UV light, alcohol, pollutants etc may act in concert for ROS generation and associated risk. Data from Irwin et.al, (2011), indicated that activation of either ERα or ERβ differentially rescued (protected) the functional efficiency of brain mitochondria, as demonstrated by increased electron transport and decreased oxidative stress.

Estrogenic upregulation of glycolytic pathways as well as maintenance of ion gradients, may lead to increased mitochondrial respiration, both in neurons and in the neuroglia that offer bio-energetic support. Activation of ERα is the primary ER initiated pathway for removal of lipid peroxide from brain mitochondria. Homeostasis of glutamatergic system is necessary during LTP enhancement by estrogen. Results with selective agonists of ERα and ERβ, indicate greater involvement of ERβ with improved neuronal respiration. ERα is prominently associated with decreased inflammation in astrocytes and ERβ in microglia. Differential activation of ERα and ERβ is required for increased mitochondrial respiration.

**IMPLICATIONS FOR HEALTH**

Vom Saal and Hughes, (2005) published list of health conditions and disabilities caused by Bisphenol A at extremely low levels, obesity in adulthood following exposure in utero. Early puberty, reduced sperm count. Prostate Disease including Tumor proliferation. Cellular causes of Miscarriage and Downs Syndrome. Increased Embryo mortality. Breast Cancer.Impaired Immune Function. Decreased level of antioxidant enzymes. Changes in Brain Chemistry. Reversal in brain Sexual Dimorphism. Changes in synapse formation in brain. Changes in Behaviour (hyperactivity,increased aggressiveness, changes in response to fear provoking or painfull stimuli. Impaired Learning. Altered sexual behavior. Decreased Maternal Behaviour. Increased Susceptibility to addiction to drugs like Amphetamine.

Undernutrition may modify risk for certain chemical induced neurologic disease and in some cases under nutrition may be prerequisite for surfacing of neurotoxicity. Neurologic disease due to under nutrition, may show similarity to neurotoxicity, especially in peripheral nervous system. In the poor, combined effect of under nutrition and neurotoxicity would be seen. In the others settings of drug, alcohol abuse, old age food faddism and medications may mix with neurotoxicants effect. Pregnancy, lactation increase nutritional demands, affecting the risk of toxicant (Spencer and Palmer, 2012). The spectrum of Bisphenol-A non-classical bioactivities is only beginning to be defined in context to human health in general and the excitable tissue biology, particularly neurophysiology. The pace of attaining such scientific understanding must match the threat posed by ubiquitous Bisphenol-A, in global environment.

**Conflict of interest statement**

There is no conflict of interest.

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