NEONATAL BISPHENOL A EXPOSURE ALTERS THYROID-AXIS AND PROOXIDANT-ANTIOXIDANT BALANCE IN BRAIN OF RAT

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ABSTRACT: *The aim of this study was to investigate the possible effects of neonatal bisphenol A (BPA) exposure on the neuroendocrine system (thyroid-brain axis). 20 or 40 µg/kg of BPA was orally administered to neonatal male albino rats (Rattus norvigicus) from postnatal days (PNDs) 15 to 30. Both administrations gave rise to a lower serum thyroxine (T4) and triiodothyronine (T3) levels, and higher thyrotropin (TSH) level than control group at PND 30. Also, a marked reduction in serum of neonatal growth hormone (GH) was observed in both treated groups. In neonatal cerebellum and cerebrum, the elevations of oxidative markers [lipid peroxidation (LPO), nitric oxide (NO), and hydrogen peroxide (H_2O_2)] due to both administrations were observed at PND 30, along with decreased activities of antioxidants markers [total ascorbic acid (TAA), total thiol (t-SH), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), and catalase (CAT)] with respect to control group. Thus, hypothyroidism in BPA might disturb the neonatal thyroid-brain axis via production of free radicals, and this, might destruct the plasma membrane and cellular components delaying cerebrum and cerebellum development.*

KEYWORDS: Bisphenol A, Thyroid hormones, Cerebellum, Cerebrum, Antioxidants, Prooxidants.

INTRODUCTION

Endocrine system regulates a developmental, metabolic, and reproductive processes including embryonic development, growth, and digestion (Ahmed et al., 2008; Ahmed, 2011, 2013 & 2016c). Endocrine-disrupting compounds (EDCs) are defined as exogenous chemicals or chemical mixtures that impact endocrine system structure or function and cause several adverse effects (Ahmed, 2016c). They may affect these processes by either binding to or blocking hormone receptors, thereby triggering or preventing hormonal response (Witorsch, 2002; Gore *et al*., 2015; Ahmed, 2016a; Ejaredar *et al*., 2016). These compounds including many agents of chemical or natural origin, among these products is bisphenol A [BPA; 2,2-bis (4-hydroxyphenyl) propane]. It is assigned as the third highest Toxicological Priority Index (ToxPi) score of the 309 chemicals examined based on its ability to interact with a number of signaling pathways by the US Environmental Protection Agency (EPA) and US National Toxicology Program (NTP) (Vandenberg *et al*., 2013). It is an industrial chemical used in thermal papers, flame retardants and the manufacture of food packaging, including polycarbonate plastics and epoxy resins lining metal cans (Johnson *et al*., 2015; Ahmed, 2016a; Sharma *et al*., 2016). The vital route of BPA exposure appears to be oral from food contact materials (Thayer *et al*., 2015), and its daily intake for adults is estimated at 0.4-1.4 mg/kg/day (World Health Organization, 2010). In human, BPA is mainly stored in the adipose tissue, with

an elimination half-life of approximately 6 h (Rochester, 2013; Leung *et al*., 2016; Muhamad *et al*., 2016).

BPA inhibits both the synthesis of thyroid hormones (THs) in the thyroid gland, and the conversion of thyroxine (T4) to its active form (T3) in peripheral tissues. The deficiency in THs (hypothyroidism) during the developmental period may result in an irreversible impairment, morphological and cytoarchitecture abnormalities, disorganization, maldevelopment and physical retardation that are permanent (Zoeller and Rovet, 2004; Zoeller and Crofton, 2005; Argumedo *et al*., 2012; Hassan *et al*., 2013). These effects can produce an increase of oxidant species that causes lipid peroxidation (LPO), nitration, carbonylation, or glutathionylation of proteins, and fragmentation of DNA (Halliwell and Gutteridge, 2007; Valko *et al*., 2007). Generally, reactive oxygen species (ROS) generation is controlled by enzymatic antioxidants such as glutathione peroxidase (GPx) and catalase (CAT), and non-enzymatic antioxidants such as total thiol (t-SH) and glutathione (GSH) (Ahmed *et al*., 2013; Al-azzawie *et al*., 2013; Imosemi, 2013). These mechanisms involve preventive mechanisms, repair mechanisms, physical and antioxidant defenses (Ahmed et al., 2014). However, studies on potential connections between BPA and neonatal brain development associated with thyroid dysfunction during the postnatal period are limited. Thus, the objective of this study was to evaluate the effect of neonatal BPA exposures on aspects of the development of the neonatal thyroid-brain axis in male albino rats (*Rattus norvigicus)* during the postnatal period, specifically at PND 30.

MATERIALS AND METHODS

- Chemicals:

Bisphenol A (BPA; 2, 2-bis(4-hydroxy-phenyl) propane) used in the present study is White to light brown flakes or powder. The chemical formula is $C_{15}H_{16}O_2$, the molecular weight is 228.29 g/mol, the CAS Registry Number is 80-05-7 and the purity is 97%. Any other chemicals were purchased at a high purity grade (99%) from Sigma company (Nasr city, Cairo, Egypt).

- Experimental animals:

A total of 24 neonatal male white albino rats (*Rattus Norvegicus*, Wistar strain) weighing about 25-35 g, were used in this investigation. They were obtained from the National Institute of Ophthalmology, Giza, Egypt. Animals were kept under observation for about 14 days before the onset of the experiment to exclude any intercurrent infections. The chosen animals were housed in plastic cages in the department of animal house at the normal atmospheric temperature (23 ± 2 °C), relative humidity 50 ± 5% and constant daily 12hr normal light/dark cycle. They were received a free access to tap water (drink *ad libitum*) and standard rodent pellet diet manufactured by an Egyptian company during the experimental period (Ahmed *et al*., 2015a,b; Ahmed, 2016b,c; Ahmed and El-Gareib, 2017). All animal procedures were in agreement with the general guidelines of animal care and the recommendations of the Canadian Council on Animal Care (Olfert et al.,1993). All efforts were made to diminish the animal suffering and to reduce the number of animals used.

- Experimental design:

BPA were dissolved in corn oil and given to neonatal rats daily and orally by gastric intubation from PND 15 to 30. Non-anesthetized neonatal rats received two doses of BPA (20 & 40 µg/kg bw/day). The vehicle control group received the same amount of corn oil during the same

period. These doses were previously reported by Ahmed (2016c). In the United States (the US Environmental Protection Agency, USEPA), Europe (the European Food Safety Authority, EFSA), and Canada (the Health Canada), regulating bodies have determined that 25-50 µg/ kg bw/day of BPA exposure is the recent tolerable daily intake (TDI) for humans, based largely on rodent multigenerational, sub-chronic, oral toxicity studies, measuring endpoints such as body weight and developmental deformations (Rochester, 2013; Kinch et al., 2015). Also, pharmacokinetic experiments, taking into account the alterations in BPA metabolism between rodents and humans have assessed that exposures to 400 µg/kg bw/day produce bioactive BPA concentrations in their blood, within the range of recorded human blood concentrations (Vandenberg et al., 2012).

At PND 30, rats were sacrificed under mild diethyl ether anesthesia in the early morning. The neonatal blood samples (6 per group) were taken from a jugular vein, allowed to coagulate at room temperature then centrifuged at 3000 round per minutes (r.p.m.) (1006.2 g) and 15-24 °C for 20 min. The clear supernatant sera were rapidly removed, divided into three portions for each individual animal, and kept at -70 °C till used. On the other hand, the slices of the selected brain regions were homogenized in 0.9% NaCl (10% w/v). The obtained homogenate was kept in deep freezer at –20 °C for measurement of prooxidant and antioxidant parameters.

- The radioimmunoassay examinations:

The serum concentrations of thyroxine (T4), triiodothyronine (T3), thyrotropin (TSH), and growth hormone (GH) were estimated quantitatively by RIA at the Diabetic Endocrine Metabolic Pediatric Unit, Center for Social and Preventive Medicine, New Childrens Hospital, Faculty of Medicine, Cairo University, Egypt, according to the method of Thakur et al. (1997) for T4, Maes et al. (1997) for T3, Mandel et al. (1993) for TSH, and Reutens (1995) for GH. The kits were obtained from Calbiotech, Inc. (Spring Valley, CA, USA).

- Biochemical assays in neonatal cerebellum and cerebrum:

The levels of lipid peroxidation (LPO), nitric oxide (NO), hydrogen peroxide (H_2O_2) were measured according to the methods of Preuss *et al.* (1998), Dutta et al. (2008), and Sergiev et al. (1997), respectively. Also, the levels of total ascorbic acid (TAA), total thiol (t-SH), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-s-transferase (GST), and catalase (CAT) were estimated according to the method of Kyaw (1978), Koster *et al.* (1986), Beutler *et al.* (1963), Pinto and Bartley (1989), Goldberg and Spooner (1983), Mannervik and Guthenberg (1981), and Cohen *et al.* (1970), respectively.

Statistical analysis:

The experimental data were analyzed with the software PC-STAT (University of Georgia, 1985). The data were evaluated by one-way analysis of variance (ANOVA) followed by LSD analysis to discern the main effects and compare various groups with each other. F-probability for each variable expresses the general effect between groups. The data were presented as mean values and standard error (SE) and the statistical differences at $P < 0.01$ and $P < 0.001$ were considered statistically highly significant and very highly significant, respectively.

RESULTS

- Neonatal thyroid and growth markers:

Both dosage administrations of BPA (20 or 40 µg/kg) to neonatal rats resulted in a highly significant increase (LSD; P < 0.01) in serum TSH level at PND 30 when compared with the control group, where the percentage indices for TSH was +121.06% in the low dose group, and +204.40% in the high dose group (Table, 1). This elevation was associated with a marked reduction (LSD; P < 0.01) in serum triiodothyronine (T3), thyroxine (T4) and Growth hormone (GH) levels at tested day with respect to the control group, where the percentage indices for T3, T4 and GH were -29.91, -33.15 and -50.51 in the low treated group and - 69.24, -51.07 and -79.08% in the high treated group respectively. Based on one-way ANOVA of these parameters, it was found that the general effect between the groups was very highly significant (P < 0.001) at examined day (Table, 1).

- Antioxidants and prooxidants markers in neonatal cerebrum and cerebellum:

In both brain regions, both administrations led to a high significant decrease in total ascorbic acid (TAA), total thiol (t-SH), and glutathione (GSH) concentrations at PND 30 when compared with the control group (Table, 2). Also, these administrations produced a highly significant decrease (LSD; P < 0.01) in the activities of catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX), and glutathione-S-transferase (GST) at studied day compared with the control group (Tables, 3 & 4).

In cerebrum, the lipid peroxidation (LPO), nitric oxide (NO) and hydrogen peroxide (H_2O_2) levels were highly significant increase in both treated groups in comparison with the corresponding control (Table, 5). Their elevations in the low dose group were +77.96% for LPO, +131.31% for NO and +200.00% for H_2O_2. These variabilities in the high dose group were +155.24, +319.44 and +571.53%, respectively. Parallelly in cerebellum, LPO, NO and H_2O_2 levels showed a highly significant increase (LSD; P < 0.01) in both treated groups, where their percentage indices in the high dose group were +154.79, +397.84 and +1021.91%, respectively or in the low dose group were +51.15, +143.96 and +384.41%, respectively when compared to control group (Table, 5).

A one-way analysis of variance (ANOVA) showed that the effect between the groups on all previous parameters was very highly significant (P<0.001) at examined day in comparison to the control group (Tables, 2-5).

DISCUSSION

In our study rats administered two different doses of BPA, the low dose which was the TDI dose and the high dose which was 10 times the TDI given daily for 30 days. Both neonatal dosage administrations of BPA affected thyroid function during postnatal period. These administrations caused a remarkable decline in the levels of neonatal serum T4, T3 and GH, and a clear increase in the level of neonatal serum TSH at PND 30 as compared to the control group. These alterations were dose dependent. Similarly, BPA caused a subclinical hypothyroidism (Wang *et al*., 2015; Porreca *et al*., 2016) by inhibiting hormone synthesis (suppressed the transcriptional activity by inhibiting T3 binding to the thyroid receptor (TR) and by recruiting N-CoR on the promoter), reducing the ability of THs to bind the transport

proteins (transthyretin) in the bloodstream (Wetherill *et al*., 2007), or increasing catabolism of THs (Evans *et al*., 2014). It potentially altered the thyrotropin-releasing hormone (TRH) that induced TSH secretion and inhibited sodium iodide symporter (NIS)-mediated iodide uptake in a concentration-dependent manner (Wu *et al*., 2016). Also, it disrupted the TRs, androgen receptor (AR), estrogen receptors (ERs), peroxisome proliferator-activated receptor-γ (PPARγ), and other endocrine-relevant signaling pathways (Wetherill *et al*., 2007). These disturbances may be a substantial risk factor for thyroid diseases (Tang *et al*., 2013). Interestingly, the reduction in the concentration of GH can be attributed to the disruption of the activities of THs (Ahmed, 2012), of the synthesis and release of growth hormone-releasing hormone (GH-RH), of the sensitivity of the pituitary gland to GH-RH, and of the transcription of the GH gene (Osfor *et al*., 2013). Based on these results, it can be suggested that the administrations of BPA might act antagonistically with developmental hypothalamic-pituitary-thyroid axis (HPTA) and this may lead to adverse developmental defects.

On the other hand, the stepwise progress in the levels of THs during this   period may be necessary for normal cerebrum and cerebellum development as observed in this study. Consistent with these data, THs in rat mainly affect cerebral and cerebellar development during the first 2-3 weeks of postnatal life (El-Bakry *et al*., 2010; Ahmed, 2012; Wang *et al*., 2012) and are required for normal maturation of these regions (Koibuchi, 2006; Bernal, 2007; Ahmed, 2011; Ahmed and Incerpi, 2013). Also, Koibuchi (2013) emphasized that the genomic and non-genomic actions of the T3 and T4 play a critical role in cerebellar development. Thus, we studied its effect on the prooxidant/antioxidant markers. In the control group, the levels of antioxidant markers (TAA, t-SH, GSH, GPx, GR, GST and CAT) and prooxidant markers (LPO, NO and H_2O_2) of neonatal cerebrum or cerebellum followed by a synchronized course of development. The enzymatic and non-enzymatic antioxidant defense variables (GR, GST, CAT, GPx, t-SH and GSH) and LPO in the control rat were substantially and gradually increased from the first to the third week old in most brain regions (Ahmed *et al*., 2006 & 2012; Ahmed, 2012). As well, the prooxidant/antioxidant balance and detoxification of potentially damaging ROS was crucial for cellular homeostasis (Livingstone, 2001; Ahmed, 2012; Ahmed *et al*., 2012), and may play an important role in a healthy life for the newborns (Ahmed *et al*., 2013). Thus, based on this evidence, higher levels of cerebellar or cerebral enzymatic and non-enzymatic antioxidant markers in the control rats detected at PND 30 may be important in protecting the neonatal brain from H_2O_2 or NO toxicity. This balance or protection may be mediated by the thyroid states of newborns. These investigations were in accordance with those of Das and Chainy (2001), Dasgupta *et al*. (2007) and Ahmed *et al*. (2008 & 2012). Also, there are modulation between THs and GSH in developing cerebrum and cerebellum of the control group. These investigations are in accordance with Ahmed *et al*. (2008 & 2010). Moreover, a positive association between the production of CAT and H_2O_2 with the THs and TSH levels in the control group. Under normal conditions, specific membrane-bound nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase (DUOX complex) provides H_2O_2 for THs synthesis (Ahmed, 2012; Petrulea *et al*., 2012), THs modulate the action of CAT on the H_2O_2 formation and breakdown (Das and Chainy, 2001), and TSH stimulates the organification of iodine by the increase in the production of H_2O_2 (Petrulea *et al*., 2012). From this, it can be inferred that the production of these markers is synergistic and closely interrelated with the behavior of the HPTA during the postnatal period. Accordingly, HPTA regulates metabolism and growth, and brain development (Lema *et al*., 2009).

In the present study, both administrations of BPA suppressed the activity of antioxidant enzymes (GPx, GR, GST and CAT) and non-enzymatic antioxidants (t-SH, TAA and GSH) in

cerebrum and cerebellum. on the other hand, prooxidant markers (LPO, NO and H_2O_2) were increased in these brain regions at PND 30. BPA caused marked oxidative impact by decreasing the activities of antioxidant enzyme compared to their activities in the control group. These results agree with the previous studies which demonstrated that administration of BPA increases MDA levels (Kabuto *et al.,* 2003). BPA administration increased dose-dependently the thiobarbituric acid-reactive substance (TBARS) levels in the brain of male rats (Aydogan *et al.,* 2010; Korkmaz *et al.,* 2011). This was indicated from the significant increase in LPO and NO level in the cortex. Chitra *et al.* (2003) and Hassan *et al.* (2013) illustrated that BPA causes cell rupture and membrane damage, and increases ROS production and oxidative stress. In similar, BPA was reported to induce oxidative damage in several tissues (Aydogan *et al.,* 2010; Korkmaz *et al.,* 2010; Korkmaz *et al.,* 2011). This results in inhibition of antioxidant enzymes (Obata and Kubota, 2000; Bindhumol *et al.,* 2003; Chitra *et al.,* 2003; Kabuto *et al.,* 2003). As well, the variations in total antioxidant defenses in developing rat brain may predispose structures to oxidative stress-related neurodegenerative disorders (Siqueira *et al.,* 2005; Kolosova *et al.,* 2006) and cell death depending on the region of the brain affected and the severity of the insult (Ferriero, 2004). The early postnatal period of development is a time of rapid change in brain architecture which shapes a broad range of neuroendocrine and characteristics. Similarly, a progressive hypothyroidism during the postnatal rat brain development led to a decline in GSH level (Rahaman *et al.,* 2001; Mogulkoc *et al.,* 2005; Ahmed, 2012), t-SH level and GPx activity (Ahmed, 2012) associated with an increase in H_2O_2 (Bhanja and Chainy, 2010) and LPO (Rahaman *et al.,* 2001; Bhanja and Chainy, 2010). These disorders are associated with augmented oxidative stress (Dasgupta *et al.,* 2005; Ahmed *et al.,* 2012) and are consistent with those reported in the present study. An alternative attribution for the present findings is that the high levels of H_2O_2 are also known to inactivate the CAT (Lardinois *et al.,* 1996) and increase tissue sensitivity to oxidant injury (Ho *et al.,* 2004). This disturbance has deleterious effect on the health of the newborns and adulthood (Ahmed *et al.,* 2013). This supports the hypothesis that decreased TH may be a relevant predictor for long-lasting developmental neurotoxicity.

CONCLUSION & FUTURE DIRECTION

Three conclusions can be drawn from these data: **(1)** Neonatal exposures to BPA seem to alter TH synthesis and secretion, either by acting directly on the thyroid gland or by acting on the pituitary or hypothalamic control of TSH or GH secretion. **(2)** These administrations appear to disrupt the prooxidant and antioxidant system. These drastic effects may play a significant role in brain and thyroid diseases. **(3)** BPA seems to play the role of a stress-responsive factor in the neonatal endocrine system (HPTA). Further investigations are required to elucidate the potential associations with human health due to the toxicity of BPA is dependent on compound congeners, dose, exposure duration, developmental period, and the species involved. From this, it can be concluded that the endocrine-disrupting compounds can exert complex, mosaic effects during an animal's life cycle (Zoeller *et al*., 2012).

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APPENDIX

Legends

Tables

Table 1. Effect of BPA on thyroid functions [triiodothyronine (T3), thyroxine (T4), and thyrotropin (TSH)] and growth hormone (GH) of neonatal rats during the postnatal period. Data are expressed as mean ± standard error. Number of animals per each group is six. Values which share the same superscript symbols are not significant. ANOVA (F-probability) expresses the effect between groups, where P < 0.001 is very highly significant.

Table 2. Effect of BPA on the levels of non-enzymatic antioxidants in neonatal cerebrum and cerebellum during the postnatal period. Data are expressed as mean ± standard error. Number of animals per each group is six. Values which share the same superscript symbols are not significant. ANOVA (F-probability) expresses the effect between groups, where P < 0.001 is very highly significant.

Table 3. Effect of BPA on the levels of enzymatic antioxidants in neonatal cerebrum during the postnatal period. Data are expressed as mean ± standard error. Number of animals per each group is six. Values which share the same superscript symbols are not significant. ANOVA (F-probability) expresses the effect between groups, where P < 0.001 is very highly significant.

Table 4. Effect of BPA on the levels of enzymatic antioxidants in neonatal cerebellum during the postnatal period. Data are expressed as mean ± standard error. Number of animals per each group is six. Values which share the same superscript symbols are not significant. ANOVA (F-probability) expresses the effect between groups, where P < 0.001 is very highly significant.

Table 5. Effect of BPA on the levels of prooxidants in neonatal cerebrum and cerebellum during the postnatal period. Data are expressed as mean ± standard error. Number of animals per each group is six. Values which share the same superscript symbols are not significant. ANOVA (F-probability) expresses the effect between groups, where P < 0.001 is very highly significant..