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Bisphenol A disrupts glucose transport and neurophysiological role of IR/IRS/AKT/GSK3β axis in the brain of male mice

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ABSTRACT

Bisphenol A (BPA), one of the most prevalent chemicals for daily use, was recently reported to disturb the homeostasis of energy metabolism and insulin signaling pathways, which might contribute to the increasing prevalence rate of mild cognitive impairment (MCI). However, the underlying mechanisms are remained poorly understood. Here we studied the effects of low dose BPA on glucose transport and the IR/IRS/AKT/GSK3β axis in adult male mice to delineate the association between insulin signaling disruption and neurotoxicity mediated by BPA. Mice were treated with subcutaneous injection of 100 μg/kg/d BPA or vehicle for 30 days, then the insulin signaling and glucose transporters in the hippocampus and prefrontal cortex were detected by western blot. Our results showed that mice treated with BPA displayed significant decrease of insulin sensitivity, and in glucose transporter 1, 3 (GLUT1, 3) protein levels in mouse brain. Meanwhile, hyperactivation of IR/IRS/AKT/GSK3β axis was detected in the brain of BPA treated mice. Noteworthily, significant increases of phosphorylated tau and β-APP were observed in BPA treated mice. These results strongly suggest that BPA exposure significantly disrupts brain insulin signaling and might be considered as a potential risk factor for neurodegenerative diseases.

1. Introduction

Bisphenol A (BPA), a kind of environmental endocrine disrupting chemicals (EDCs), is present ubiquitously in our lives. It is used commercially in products containing polycarbonate plastics such as food and drink packaging materials and infusion bags which sometimes may be boiled, microwave heated, autoclaved and brushed (Vandenberg et al., 2009). It has been reported that more than 90 percent of the population aged above six in the USA has detectable levels of BPA in urine and blood (Taylor et al., 2011). Since BPA is rapidly metabolized, it suggests that human exposure to BPA might be continuous and *via* multiple sources, such as inhalation and contact, not only limited for ingestion (Stahlhut et al., 2009). The detection of adverse effects in numerous of animal models upon exposure to environmentally relevant doses of BPA that correspond to those observed in humans, strongly supports that the endocrine disrupting activities of BPA contribute to adverse effects on human health.

Recently, the adverse effects of BPA on glucose homeostasis have been demonstrated in numerous studies. Glucose-stimulated insulin secretion (GSIS) and serum insulin were found to be elevated upon exposure to 100 μg/kg/day BPA, suggesting the direct effect of BPA on pancreatic β-cell function (Nadal et al., 2009). Furthermore, BPA affects hormone signaling and causes endocrine dysfunction by binding to estrogen receptors and promotes both agonist and antagonist activity (Pan et al., 2013). Also it has been shown to cause persistent aberrations in spontaneous behavior and in learning and memory in rodents (Yang et al., 2014). Several investigations have been undertaken to explore the neurotoxic effects of BPA, especially in fetal brain development and its promotion of neurodegenerative diseases. *In vitro* studies support that BPA causes adverse neurological effects as it induces impairments in dendritic and synaptic development in cultured fetal rat hypothalamic cells (Leranth et al., 2008) and inhibits neurite extension in rat pheochromocytoma (PC12) cells (Seki et al., 2011). Furthermore, BPA may act as a DNA methylation agent to alter gene expression in the rodent brain, which may be a plausible reason for the developmental neurotoxic effects (Wolstenholme et al., 2011).

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With global aging, the prevalence of obesity, type 2 diabetes and neurodegenerative diseases have been astonishingly increasing. These diverse pathologies shared the same characteristic, insulin resistance. Therefore, the role of insulin in human health has become more and more critical and may be relevant to the age-related disorders, which are associated with disturbances in glucose metabolism. Noteworthily, the brain was once considered to be an insulin insensitive tissue. However, Havrankova et al. indicated the widespread presence of insulin receptors (IRs) and higher levels of insulin in the central nervous system (CNS) than periphery (Havrankova et al., 1978), where it plays a critical physiological role. In the past decade, growing evidence from *in vivo* and *in vitro* studies confirms that insulin and its receptor, can affect nervous physiology, including energy homeostasis and cognitive processes (de la Monte, 2012). As in peripheral tissues, insulin signaling starts with binding to the IR, which is activated by auto tyrosine phosphorylation. IR then phosphorylates insulin receptor substrate (IRS) proteins, and the activated IRSs serve as docking sites to activate the downstream signals such as (protein kinase B) AKT/PKB and glycogen synthase kinase 3 beta (GSK-3β), which play crucial roles in the development of neuronal structure (Chiu and Cline, 2010). Therefore, understanding of the link between insulin signaling axis disruption and neurotoxicity could provide potential therapeutic strategies targeting BPA-mediated neurotoxicity.

Our previous work suggested that BPA contributed to endocrine dysfunction by interfering insulin biosynthesis and secretion (Liu et al., 2013). Based on the work, the present study was undertaken to assess its deregulation of the IR/IRS/AKT/GSK3β axis, which might represent a key-contributing factor to the neurodegenerative process that culminates in Alzheimer-like dementia.

2. Materials and methods

2.1. Animals and treatment

All experiments involving animals and tissue samples were conducted in accordance with the guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) (USA), and all procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of Nanjing Medical University (China). The C57BL6 male mice, 7–8 weeks old and 25–30 g weight, were purchased from the Model Animal Research Center of Nanjing University (Nanjing, China). The mice were housed throughout the experiments under specific pathogen free (SPF) conditions, with controlled illumination (12 h light/12 h dark cycles), humidity (30–50%), and temperature (18–22 °C).

Adult mice were randomly assigned to receive 50ul corn oil (vehicle) or BPA (100 μg/kg/day; Sigma–Aldrich) for 30 days. Subcutaneous injection was chosen for the present study to mimic the nonfood sources of BPA exposure. Then, 24 h after the last dosing, the animals were sacrificed by cervical dislocation. The brain was rapidly removed, and the hippocampus and frontal cortex were immediately dissected and frozen at −80 °C for further experiment.

2.2. Glucose and insulin tolerance tests

For intraperitoneal glucose tolerance tests (IPGTT), after 30 days of treatment with BPA, animals were fasted overnight for 16 h, and then intraperitoneally injected with glucose at 2 g/kg body weight. Blood samples were collected from the angular vein and measured for blood glucose levels at 0, 15, 30, 60 and 120 min. For the intraperitoneal insulin tolerance tests (IPITT), 6-hour-fasted animals were injected intraperitoneally with 1 IU/kg body weight soluble insulin. Blood glucose was measured at the time points of 0, 15, 30, 60 and 120 min after insulin administration using an Accu-Check compact glucometer (Roche, Madrid, Spain).

2.3. Western blot analysis

Tissues were homogenized with the help of ultrasonic cell disrupter in 200 μL of cold RIPA lysis buffer (Sigma–Aldrich) with protease inhibitor. The homogenates were centrifuged at 12,000×*g* for 10 min at 4 °C and supernatants were measured using a BCA Protein Assay Kit. For western blots, 50 μg of proteins were resolved with 10% SDS-PAGE and transferred to PVDF membranes (Millipore). The membranes were blocked in 5% nonfat dried milk. Blots were then incubated with the primary antibodies at 4 °C overnight. After washing, membranes were incubated at room temperature with anti-mouse or anti-rabbit horseradish peroxidase-conjugated IgG (1:50,000). Immunoreactivity was detected using an enhanced chemiluminescence reaction (Millipore). Densitometrica nalysis employed Image J (NIH, USA). The following primary antibodies (Cell Signaling Technology or proteintech, China) and dilutions were used: anti-insulin (15848-1-AP, 1:2000), anti-p-IR Tyr1355 (#3024, 1:1000), anti-IR (1:2000, #3025), anti-p-IRS1 (1:500, BS4835), anti-IRS1 (1:500, #2382), anti-p-AKT Ser473 (1:2000, #4060), anti-AKT (1:2000, #4691), anti-p-GSK3β (1:1000, #D85E12), anti-GSK3β (1:2000, #27C10), anti-GLUT1 (1:1000, 21829-1-AP), anti-GLUT3 (1:1000, 20403-1-AP), anti-GLUT4 (1:2000, ab18831), anti-GAPDH (1:10,000 dilution; Sigma–Aldrich).

2.4. Statistical analysis

All data were normalized and expressed as mean ± SEM. The unpaired Student's *t* test and repeated-measures one-way ANOVA were used as appropriate for comparison between groups of mice. Statistical significance was assumed at $p < 0.05$.

3. Results

3.1. BPA decreases insulin sensitivity and plasma insulin level in adult mice

The IPGTT and IPITT were performed to investigate the effect of BPA on glucose homeostasis and insulin sensitivity. As depicted in Fig. 1A, BPA exerted no obvious effect on blood glucose levels, and the AUC of glucose in each group displayed no significant difference (Fig. 1A). However, BPA treated mice showed decreased insulin sensitivity in comparison to control mice when insulin tolerance tests were performed ($p < 0.05$; Fig. 1B), and the corresponding AUC was significantly increased in BPA-treated mice ($p < 0.05$).

3.2. BPA impairs IR/IRS/AKT/GSK3β axis in the hippocampus and prefrontal cortex

Since peripheral insulin homeostasis is closely associated with the insulin signaling IR/IRS/AKT/GSK3β axis, the next step was to ascertain whether BPA disrupts this signaling axis in the brain. Our data indicated that insulin levels in both hippocampus ($p < 0.05$) and prefrontal cortex ($p < 0.01$) were obviously increased in BPA-treated mice. Similarly, the phosphorylated IR (Tyr1355) as well as the downstream signal protein phosphorylated IRS1 (Tyr896 and Ser307) were significantly increased in both hippocampus ($p < 0.05$) and prefrontal cortex ($p < 0.01$) in BPA-treated mice (Fig. 2). Since AKT plays a crucial role in insulin signaling, we then assessed the expression of this signal protein. As shown in Fig. 3, the phosphorylated AKT (Ser473) was obviously increased in hippocampus ($p < 0.01$) and prefrontal cortex ($p < 0.05$) in BPA-treated mice. In parallel with the effect of BPA on AKT phosphorylation, BPA