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Altered heart proteome in fructose-fed Fisher 344 rats exposed to bisphenol A

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ABSTRACT

Bisphenol A (BPA), is an artificial estrogen initially produced for medical purposes but is today widely used in polycarbonate plastics and epoxy resins. Exposure-related reproductive disorders have been found, but recently it has also been suggested that BPA may be involved in obesity, diabetes, myocardial hypertrophy and myocardial infarction in humans.

To mimic a modern lifestyle, female rats were fed with fructose or fructose plus BPA (0.25 mg/L drinking water). The myocardial left ventricle proteome of water controls, fructose-fed and fructose-fed plus BPA supplemented rats was explored. The proteome was investigated using nano-liquid chromatography tandem mass spectrometry and two-dimensional gel electrophoresis followed by matrix assisted laser desorption/ionization mass spectrometry identification.

In total, 41 proteins were significantly altered by BPA exposure compared to water or fructose controls. Principal component analysis and cellular process enrichment analysis of altered proteins suggested increased fatty acid transport and oxidation, increased ROS generation and altered structural integrity of the myocardial left ventricle in the fructose-fed BPA-exposed rats, indicating unfavorable effects on the myocardium.

In conclusion, BPA exposure in the rats induces major alterations in the myocardial proteome.

1. Introduction

Endocrine disruptors, is a family of chemicals with the ability to affect the endocrine system in a detrimental manner that may contribute to a number of diseases (Casals-Casas et al., 2011; Schug et al., 2011; Melzer et al., 2010). Bisphenol A (BPA) is a member of the bisphenol family that also includes structural analogues such as Bisphenol B, Bisphenol F and Bisphenol S (Rosenmai et al., 2014). BPA was initially manufactured as a potential synthetic estrogen for preventing miscarriage and premature births in the 1930s but has since 1940s been used as a softener in plastics (Rubin, 2011). Due to incomplete polymerization or degradation of the polymers, BPA may leach out into the surrounding environment (Carlwile et al., 2009). Confirming the relevance of these concerns, studies have shown that BPA is widespread in the general population (Peretz et al., 2014) and that more than 90% of the US population have detectable levels in their urine (Calafat et al., 2008). BPA does not belong to the group of persistent pollutants and clearance routes are conjugation of BPA into BPA-glucuronide in the liver followed by excretion via bile (Inoue et al., 2004) or via urine (Völkel et al., 2002), however, since a majority of the human population is exposed to BPA daily, the exposure and presence in our bodies could be regarded as of persistent character.

An increasing amount of data indicates adverse health effects of BPA exposure. It has since long been known that BPA can interact with biologically important hormone receptors (Gould et al., 1998; Matthews et al., 2001; Jung et al., 2007) and the role of BPA in reproductive health has recently been highlighted (Mathieu-Denoncourt et al., 2015; Peretz et al., 2014). Interestingly, BPA has also, together with other environmental pollutants such as polychlorinated biphenyls and heavy metals, been reviewed in

Abbreviations: 2DE, two-dimensional gel electrophoresis; BPA, Bisphenol A; FA, Formic acid; FDR, False discovery rate; DHB, 2,5-dihydroxybenzoic acid; MALDI-TOF, matrix associated laser desorption/ionization time-of-flight; MS, mass spectrometry; MS/MS, tandem mass spectrometry; nLC, nano-liquid chromatography; S1P, sphingosine-1-phosphate; TFA, Trifluoroacetic acid.

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relation to weight regulation (Kelishadi et al., 2013). Metabolic alterations linked to BPA exposure have been found in mice later in life (Marmugi et al., 2012) and after perinatal exposure to BPA in combination with high fat diet in rats later in life (Wei et al., 2011). In addition, our group has recently shown that BPA exposed fructose fed juvenile female rats have increased circulating apo A-I levels and increased liver somatic index (Rönn et al., 2013b).

Epidemiological studies indicate a correlation between high levels of urinary BPA in human adults and increased incidence of cardiovascular complications (Melzer et al., 2010; Lind and Lind, 2011; Gao and Wang, 2014). The link between BPA and CVD is a highly relevant topic and has recently been summarized by Ranciere et al. (2015). In experimental studies BPA related pro-arrhythmic effects in female rat cardiac cells have been found (Gao et al., 2013). Regarding cardiotoxicity, Aboul Ezz et al. (2015) showed that male BPA exposed rats generated reactive oxygen species and had reduced anti-oxidative capability in their heart tissue, alterations that may indicate mitochondrial dysfunction. Interestingly, and supporting the study by Aboul Ezz et al., liver mitochondrial dysfunction as result of prenatal BPA exposure of rats have been reported by Jiang et al. (2014). Further indicating cardio-toxic effects of BPA, maternal BPA exposure has been shown to impact the fetal heart transcriptome in monkeys, affecting genes involved in cardiac hypertrophy (Chapalamadugu et al., 2014) and in line, Patel et al. have found left chamber hypertrophy in mice as result of BPA exposure (Patel et al., 2013).

In order to study the impact of BPA exposure on heart structure and function, our aim was to investigate myocardial metabolism, more particularly the myocardial left ventricle proteome in the BPA exposed fructose fed juvenile female rats described above (Rönn et al., 2013b) compared to water and fructose controls. Two different untargeted approaches were applied; nano-liquid chromatography (nLC) tandem mass spectrometry (MS/MS) and two-dimensional gel electrophoresis (2-DE) for protein separation and quantification followed by matrix associated laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) for identification of significantly altered proteins.

2. Methods

2.1. Animals

As described by Rönn et al. (2013b) the animal study was approved by the Uppsala Animal Ethical Committee and did follow the guidelines by the Swedish Legislation on Animal Experimentation (Animal Welfare Act SFS1998:56) and European Union Legislation (Convention ETS123 and Directive 86/609/EEC). In the main study, sixty female F 344 rats at 3 weeks of age were purchased from Charles River International, Salzfeld, Germany, and housed 3 rats/cage at Uppsala University Hospital animal facility, in a temperature-controlled and humidity-controlled room with a 12-h light/dark cycle. To minimize background BPA exposure Polysulfone IV cages (Eurostandard IV) and glass water bottles were used. The rats were fed a standard pellet RM1 diet (ad lib.) from NOVA-SCB, Sollentuna, Sweden. During the two-week acclimatization period preceding the ten-week intervention all animals were given water to drink and during the intervention water or 5% fructose solution. At 5 weeks of age the rats were assigned to five groups (12 rats/group); water control(W), fructose control (F), low dose BPA (0.025 mg/L), medium dose BPA (0.25 mg/L) or high dose BPA (2.5 mg/L). To avoid unnecessary stress no cage-mates were separated, but the cages were allocated to the different groups to achieve equality in weights in all groups. Food and liquid consumption in each cage and individual weight of the rats were determined once a week. Before MRI exam, the rats were anesthetized with Ketalar 90 mg/kg body weight (Pfizer, New York, NY) and Rompun 10 mg/kg bw (Bayer, Leverkusen, Germany). Immediately after the scanning they were killed by exsanguinations from the abdominal aorta while still under anesthesia.

2.2. Exposure

To prepare BPA exposure solutions (0.025, 0.25 and 2.5 mg/L), three stock solutions of BPA in 1% ethanol (2.5 mg/L, 25 mg/L and 250 mg/L) were diluted 1:100 in 5% fructose solution. The low dose was chosen to be well below the recommended TDI, the medium dose corresponding to TDI (50 g/kg and day), while the highest dose was ten times this level. BPA was analyzed by liquid chromatography–MS/MS by the Division of Occupational and Environmental Medicine in Lund, Sweden which is a European reference laboratory in the DEMOCOPHES EU project (www.eu-hbm.info/democophes) for analysis of BPA. The BPA concentrations in analyzed samples of the solutions were: water control—0.00020 mg/L; fructose control—0.00011 mg/L; BPA 0.025 mg/L—0.029 mg/L; BPA 0.25 mg/L—0.25 mg/L and BPA 2.5 mg/L—2.7 mg/L. The exposure solutions were given ad lib. for ten weeks. The water control rats and the fructose control rats had free access to water containing 1% ethanol, and 5% fructose solution containing 1% ethanol, respectively. Groups given fructose solution drank more than the water control rats, and also raised their liquid consumption during the experiment, but ate less. The control group given water had an almost constant food and liquid intake.

2.3. Sample preparation

In the present pilot study, controls (n = 3 + 8), fructose-fed (n = 3 + 8) and fructose-fed BPA administered (0,25 mg/L) (n = 3 + 9) rats were included for 2-DE analysis and nLC-MS/MS respectively. Dissected rat heart left ventricles were immersed in liquid nitrogen and crushed with mortar and pestle. For two-dimensional gel electrophoresis (2-DE), sample pieces were transferred into 2-DE sample solution (Görg et al., 2000) and protein concentrations were measured with 2D Quant Kit (GE Healthcare, Little Chalfont, United Kingdom). For nLC-MS/MS experiments, samples were transferred to an 8 M Urea and 2 M Thiourea solution in 25 mM ammonium bicarbonate buffer containing 1 mg/mL anti-protease solution (Pefabloc, Roche, Switzerland). The solution was homogenized by sonication 3 × 10 s and kept at 4° for 1 h to allow proteins to denature. Samples were centrifuged at 40,000*g* for 10 min and supernatants were transferred to new tubes. Protein concentrations were measured with 2D Quant Kit (GE Healthcare) and samples were kept at −80° until analysis.

2.4. 2-DE

For 2-DE, heart left ventricle from three rats from each group were randomly selected for analysis. Samples were thawed and separated according to previous publications (Karlsson et al., 2005). In short, 200 μg of proteins were loaded into and separated in an IPG Dry Strip 3-10NL (GE Healthcare) according to isoelectric point. The strip was then transferred onto a homogenous SDS-PAGE gel (C = 14%, T = 1.5%) and separated according to size. Gels were silver stained according to Shevchenko et al. (1996) and proteins were visualized using a Versa doc system (Bio-Rad, Hercules, USA). The relative amount of each protein was normalized and expressed as percent of total gel staining by using the PDQuest software (version 4.0, Bio-Rad).

2.5. MALDI-TOF MS

Altered proteins on the 2-D gels were excised and analyzed in a MALDI-TOF mass spectrometer (Voyager DE-PRO, Applied