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Gender-dependent and genotype-sensitive monoaminergic changes induced by polychlorinated biphenyl 153 in the rat brain

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ABSTRACT

Polychlorinated biphenyls (PCBs) are present as *ortho*- and non-*ortho*-substituted PCBs, with most of the *ortho*-substituted congeners being neurotoxic. The present study examined effects of the *ortho*-substituted PCB 153 on dopamine, serotonin and amino acid neurotransmitters in the neostriatum of both male and female Wistar Kyoto (WKY) and spontaneously hypertensive rat (SHR) genotypes. PCB 153 exposure at p8, p14 and p20 had no effects on levels of these transmitters when examined at p55, but led to increased levels of both homovanillic acid and 5-hydroxyindoleacetic acid, the degradation products of dopamine and serotonin, respectively, in all groups except the female SHR. Immunoblotting showed that PCB exposure induced gender-specific decreases in dopaminergic synaptic proteins. These included a novel finding of decreased levels of the dopamine D5 receptor in both genders and genotypes, whereas male-specific changes included decreases in the postsynaptic density (PSD)-95 protein in the WKY and SHRs and a decrease in the presynaptic dopamine transporter in both the WKY and, less clearly in the male SHR. A female-specific tendency of increased vesicular monoamine transporter-2 was observed in the SHRs after PCB exposure. No changes were seen in tyrosine hydroxylase, the cytoskeletal neurotubulin or the plasma membrane marker Na^+/K^+-ATPase in any strain. Hence, PCB-exposure led to increases in monoamine transmitter turnover in both male and female animals, whereas decreases in both pre- and postsynaptic dopaminergic proteins were predominantly seen in male animals. PCB 153 may therefore induce neostriatal toxicity through both presynaptic and postsynaptic mechanisms in both genotypes and genders, including effects on the aspiny interneurons, which employ the D5 receptor to mediate dopamine effects on interneurons in the basal ganglia.

1. Introduction

Polychlorinated biphenyls (PCBs), persistent industrial contaminants that are widely distributed in the environment (Kodavanti and Tilson, 1997; Mariussen and Fonnum, 2006), can be separated into *ortho*- and non-*ortho*-substituted PCBs, with most of the *ortho*-substituted being neurotoxic. These lipophilic compounds enter the human body both prenatally through the placenta and postnatally through breast milk and fatty food (Safe, 1994). PCBs may be associated with behavioral and cognitive dysfunctions in both humans, non-human primates and rodents (Chevrier et al., 2008; Johansen et al., 2011, 2014; Schantz et al., 2003).

Underlying neurobiological mechanisms of PCBs may include changes in neurotransmission and synaptic plasticity, Ca^{2+}-dynamics and oxidative stress responses (Mariussen and Fonnum, 2006; Seegal et al., 2010). In particular, several PCBs may induce strong inhibition of neurotransmitter transporters, including the Na^+-dependent plasma membrane dopamine (DA) and serotonin (5-HT) transporters (DAT and SERT, respectively) and the H^+-dependent vesicle monoamine transporter-2 (VMAT-2) (Mariussen and Fonnum, 2001; Seegal et al., 2010; Wigestrand et al., 2013). Such effects may change interactions between e.g., monoaminergic and amino acidergic transmitter systems, specifically those mediated by neurons employing DA or 5-HT and those using

Abbreviations: 5-HIAA, 5-hydroxyindole-3-acetic acid; 5-HT, serotonin; DA, dopamine; DAT, dopamine transporter; DHBA, 3,4-hydroxybenzylamine; HPLC, high performance liquid chromatography; HVA, homovanillic acid; PCB, polychlorinated biphenyl; p, postnatal day; PSD-95, postsynaptic density protein 95; SERT, serotonin transporter; SHR, spontaneously hypertensive rats; TH, tyrosine hydroxylase; VMAT-2, vesicular monoamine transporter-2; WKY, Wistar Kyoto.

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glutamate as transmitters (Arnsten et al., 2012; Lisman and Grace, 2005). This kind of interaction may be linked to a number of behavioural and psychiatric dysfunctions, including the hypodopaminergic attention deficit hyperactivity disorder (ADHD) (Russell, 2003). This condition is characterized by hyperactivity, impulsiveness and inattention, and usually occurs with onset in early childhood, but may also persist into adulthood (Biederman and Faraone, 2005). The PCBs represent one type of environmental factor that may contribute, either alone or in concert with a genetic vulnerability, to the prevalence of ADHD (Williams and Ross, 2007; Mariussen and Fonnum, 2001; Holene et al., 1998).

Recent studies have reported effects of PCB 153 on the behavior of spontaneously hypertensive rats (SHRs) obtained from Charles River and the Wistar Kyoto (WKY) strain obtained from Harlan, UK, which are genetically related (DasBanerjee et al., 2008; Johansen et al., 2011, 2014). At the present time, the SHRs appear to constitute the best validated animal model of ADHD, combined subtype (Sagvolden and Johansen, 2012). These data indicated that postnatal exposure to PCB 153 led both genders from these two strains, which are genetically related (DasBanerjee et al., 2008), to develop distinct behavioral changes, as determined by an operant procedure which measured sustained attention, hyperactivity and impulsivity. The present study has investigated whether such PCB 153 exposure also led to differences in monoamine or amino acid transmitter dynamics in the brain. For this purpose, high performance liquid chromatography (HPLC) analysis was performed on extracts from the neostriatum in order to determine levels of neurotransmitters and their metabolites in a way which allowed us to calculate turnover ratios for, e.g., the monoamines DA and 5-HT as well as the amino acid transmitter Glu (Dervola et al., 2012). Moreover, a detailed immunochemical analysis of the proteins in nigrostriatal DA synapses in the PCB 153-exposed and control animals was performed. The data indicate that the PCB 153 congener induced enhancements of DA and 5-HT turnover in all male rats, whereas only the WKY responded in the female. Moreover, the levels of a distinct set of proteins in dopaminergic synapses were affected, one of which responded in both sexes, while others responded specifically in the male, whereas the DA synaptic proteins in the female rats were only weakly affected.

2. Methods

2.1. Animals and PCB exposure

Wistar Kyoto (WKY/NHsd) and spontaneously hypertensive (SHR/NCrl) rats were bred at the Norwegian Defense Research Establishment (Kjeller, Norway). The separate dams were kept under standard laboratory animal conditions (22 °C, 55% humidity and 12 h light/dark cycle) in type IV macrolon cages and aspen bedding, with free access to food (RM (E), Special Diet Services, Witham Essex, UK) and water. Offsprings of both genders were exposed to corn oil without or with 6 mg PCB 153 (2,2′, 4,4′, 5,5′-hexachlorobiphenyl) per kg body weight during the lactational period. PCB 153 dissolved in corn oil was given orally through a plastic tube.

To avoid acute toxicity, PCB was administered at postnatal days p 8, p14 and p20, in a total volume of 0.01 ml/g body weight for each exposure, and was given during the lactational period because this period has been reported to have a more profound PCB-induced impact on gene expression than, e.g., during the gestational period (Sazonova et al., 2011). Procedures and experiments were conducted in accordance with Norwegian laws and regulations, and were approved by the Norwegian Animal Research Authority (NARA). The PCB 153 preparation used in this study (total 6 mg/kg body weight) was specially purified and free from dioxin-like PCBs (a gift from Dr. Patrik Anderson, University of Umeå, Sweden).

Sample collection: At p55-60, both control and PCB-treated WKY and SHR rats of both genders were stunned and rapidly decapitated. The neostriata were removed, frozen in liquid N_2, and stored at −70 °C for later sample preparation and analyses.

2.2. Neurochemical analysis of neostriatum

One neostriatum from each animal was homogenized in a glass/Teflon Potter-Elvehjem homogenizer by hand in 30 strokes, with 200 μl of a buffer containing 1 mM EDTA, 10 mM Hepes and 1× protease inhibitor (Complete cocktail from Roche). Half of the homogenate was transferred to HPLC analysis, while the other half, used for western analysis, was added to a buffer containing 1 mM EDTA, 10 mM Hepes, 50 mM NaCl, 1% SDS and 1× protease inhibitor cocktail (Roche). Homogenates were stored at −70 °C until use. Solutions were made with purified 18Ω/cm H_2O (Milli-Q Advantage A10, Millipore).

2.2.1. Immunoblotting analysis

The analysis was performed as described (Bogen et al., 2006) with $n = 4$ animals for each group. Antibodies used for blotting included rabbit primary antibodies against tyrosine hydroxylase (TH) AB151 (diluted 1:20 000 in 5% bovine serum albumin (BSA), secondary antibody contained 5% dry-milk) from Chemicon International (Billerica, United States), DAT (H-80) SC14002 (diluted 1:2000 in 0.5% BSA) from Santa Cruz (Heidelberg, Germany), postsynaptic density protein, 95 kDa (PSD-95, SAP-90, DLG 4) 124 002 (diluted 1:6000 in 0.5% BSA) from Synaptic Systems (Göttingen, Germany), dopamine D1 receptor SC14001 (diluted 1:300 in 1% BSA, secondary antibody contained 0.5% dry-milk) from Santa Cruz (Heidelberg, Germany), dopamine D5 receptor 20310-1-AP (diluted 1:4000 in 0.5% BSA) from Proteintech (Manchester, United Kingdom), and VMAT-2 138302 (diluted 1.5:10 000 in 1% BSA) from Synaptic Systems (Göttingen, Germany). The mouse primary antibodies had been raised against neurospecific tubulin (TUJ1) MMS-435-P (diluted 1:40 000 in 2.5% dry-milk, secondary antibody contained 1.25% dry-milk) from Nordic Biosite (Oslo, Norway), or Na^+/K^+-ATPase, ab7671 (diluted 1:5000 in 0.5% BSA) from Abcam (Cambridge, United Kingdom). Secondary anti-rabbit Ig horseradish peroxidase and anti-mouse Ig horseradish peroxidase were obtained from Amersham Biosciences (Buckinghamshire, UK), and both were diluted 1:10 000 in the presence of either BSA or dry-milk as noted above, before incubation. Testing of the different antibodies included dilution curves with 2.5, 5, 7.5 and 10 μg neostriatal proteins.

2.2.2. Neurotransmitter analysis

Monoamine and amino acid analyses were performed with HPLC. L-Amino acid standards, including aspartic acid (aspartate), glutamic acid (glutamate), serine, glutamine and glycine were obtained from Pierce (Rockford, IL, USA). Taurine, γ-amino butyric acid, and α-amino adipic acid as well as the monoamine standards dopamine (DA), homovanillic acid (HVA), serotonin (5-HT), 5-hydroxyindole-3-acetic acid (5-HIAA) and 3, 4-hydroxybenzylamine (DHBA), as well as $HClO_4$ and ascorbic acid were from Sigma–Aldrich (Steinheim, Germany). The BCA-assay kit (Thermo-Scientific, Rockford, USA), as well as *n*-hexane (Merck, Darmstadt, Germany) were from VWR. Solutions were made with purified 18 MΩ/cm H_2O (Milli-Q Advantage A10, Millipore).

2.2.3. Extract preparation

Homogenates for HPLC-analysis were prepared as described above (with $n = 7–10$ animals within each group). Samples were thawed, and aliquots were mixed with ice cold $HClO_4$ (0.2 M) to