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Chronic treatment with polychlorinated biphenyls (PCB) during pregnancy and lactation in the rat
Part 2: Effects on reproductive parameters, on sex behavior, on memory retention and on hypothalamic expression of aromatase and 5alpha-reductases in the offspring

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ABSTRACT

The gender-specific expression pattern of aromatase and 5alpha-reductases (5alpha-R) during brain development provides neurons the right amount of estradiol and DHT to induce a dimorphic organization of the structure. Polychlorinated biphenyls (PCBs) are endocrine disruptive pollutants; exposure to PCBs through placental transfer and breast-feeding may adversely affect the organizational action of sex steroid, resulting in long-term alteration of reproductive neuroendocrinology. The study was aimed at: a) evaluating the hypothalamic expression of aromatase, 5alpha-R1 and 5alpha-R2 in fetuses (GD20), infant (PN12), weaning (PN21) and young adult (PN60) male and female rats exposed to PCBs during development; b) correlating these parameters with the time of testicular descent, puberty onset, estrous cyclicity and copulatory behavior; c) evaluating possible alterations of some non reproductive behaviors (locomotion, learning and memory, depression/anxiety behavior). A reconstituted mixture of four indicator congeners (PCB 126, 138, 153 and 180) was injected subcutaneously to dams at the dose of 10 mg/kg daily from GD15 to GD19 and then twice a week till weanling. The results indicated that developmental PCB exposure produced important changes in the dimorphic hypothalamic expression of both aromatase and the 5alpha-Rs, which were still evident in adult animals. We observed that female puberty onset occurs earlier than in control animals without cycle irregularity, while testicular descent in males was delayed. A slight but significant impairment of sexual behavior and an important alteration in memory retention were also noted specifically in males. We conclude that PCBs might affect the dimorphic neuroendocrine control of reproductive system and of other neurobiological processes.

Introduction

Polychlorinated biphenyls (PCBs) are a group of widespread environmental endocrine disrupting compounds consisting in more than 200 congeners with a broad spectrum of biologic and toxic activities (La Rocca and Mantovani, 2006). Developmental exposure to these pollutants, through placental and breast milk transfer (Safe, 1990), may interfere with the sex-specific pattern of brain development of the offspring, inducing permanent changes of the neuroendocrine control of reproduction (Kaya et al., 2002; Chung et al., 2001; Steinberg et al., 2007). Besides changes in reproductive neuroendocrinology, PCBs may also induce subtle and long-lasting neurological damages resulting in the impairment of motor and learning abilities (Roegge and Schantz, 2006; Faroon et al., 2001b; Piedrafita et al., 2008; Ulbrich and Stahlmann, 2004). Many PCBs behave as agonists/antagonists of the sex steroid hormone receptors (Janosek et al., 2006; Schrader and Cooke, 2003) and inhibit aromatase activity (Hany et al., 1999; Woodhouse and Cooke, 2004; Wojtowicz et al., 2005; Colciago et al., 2006); moreover, coplanar congeners are agonists of the arylhydrocarbon receptor (AhR) (Maier et al., 2007). The presence and progressive increase of AhR expression in the perinatal hypothalamus of both sexes (Pravettoni et al., 2005) suggest a physiological role of this receptor also in brain development. It is well known that PCBs may also modify neurotransmitter levels in various brain areas, as well as their synaptic uptake "in vitro" (Faroon et al., 2001a; Khan and Thomas, 2004; Mariussen and Fonnum, 2006).

The dimorphic ability of the adult central nervous system (particularly the hypothalamus) to respond to hormonal and environmental inputs is acquired during a critical period (from the last third of gestation to infancy), when a complex series of "organizational" events induces a dimorphic differentiation of specific neuronal networks (see

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Negri-Cesi et al., 2004 and Morris et al., 2004 for references). Puberty is the second developmental period during which further hormonal-driven refinement of neural circuits results in the ultimate establishment of the dimorphic adult brain functions (Sisk and Zehr, 2005). The main differentiating signal for the perinatal hypothalamus arises from different blood levels of testosterone (T) between males and females (Weisz and Ward, 1980; Corbier et al., 1978); in many instances T should be locally converted into active metabolites: estradiol, which then acts through the two estrogen receptors (ERalpha and ERbeta), or DHT, which binds to the androgen receptor (AR) with an affinity higher than that of the native hormone. The enzymes involved are the aromatase and the two 5alpha-reductases (5alpha-R1 and 5alpha-R2), respectively. Even though the two 5alpha-Rs catalyze the reduction of the delta 4–5 double bound of all the 3keto-delta4-steroids (androgens, progestagens and corticosteroids), these isozymes possess different kinetic properties and distinct physiological functions. 5alpha-R1, which has a low affinity for T and the other 3keto-delta4-steroids, is widely expressed in the brain (Poletti et al., 1997), and has a catabolic role (Russel and Wilson, 1994). On the contrary, 5apha-R2, which possesses a high affinity for the hormones, is mainly concentrated in the peripheral androgen-dependent structures and in the developing brain (Poletti et al., 1997) and is considered the isoform specific for the activation of T in males (Russel and Wilson, 1994).

Thus, the relative amounts of these enzymes in the differentiating hypothalamus of males and females (see Colciago et al., 2005; Poletti et al., 1998b and references herein), along with differences in AR and ER levels (Negri-Cesi et al., 2007), amplify and differentiate the action of T, dictating the dimorphic evolution of the structure.

It is well known that T aromatization is critical for the perinatal brain masculinization, at least in rodents (see Celotti et al., 1992 and Negri-Cesi et al., 2008 for references); the function of aromatase in the female brain development, as well as later in life, is less clear. Some data indicate that increased activity of the enzyme is necessary, in male and/or female hypothalamus, in selected periods of the reproductive life: onset of female puberty (Tian et al., 2004), organization of the central feedback mechanisms and of adult adaptive responses during youth (Kellogg and Lundin, 1999; Roselli, 2007), activation of male sexual behavior (Lephart, 1996), and neuroprotection (Veiga et al., 2004).

Some roles for 5alpha-R2 in the dimorphic evolution of both the prenatal (Yonehara et al., 2003; Goto et al., 2005; Ribeiro and Pereira, 2005) and pubertal brain (Ciofi et al., 2007), as well as in the control of adult hypothalamic-pituitary axis (Poletti et al., 2001) and aggressiveness have been proposed; the function of this enzyme in females is still unknown. Based on defects observed in animals knocked out for each or both 5alpha-Rs, it has been proposed that 5alpha-R1 has catabolic/anabolic role important in female reproduction (particularly during pregnancy and delivery), while the anabolic function of 5alpha-R2 is essential for male reproductive physiology (Mahendroo and Russell, 1999). However, recent findings have suggested a possible participation of both isozymes also in the development and maintenance of sexually dimorphic structures of females (Torres and Ortega, 2006).

Although the huge amount of literature available (reviewed in Negri-Cesi et al., 2007 and Ulbrich and Stahlmann, 2004), it is still difficult to draw a clear picture of the long-term reproductive and neurobehavioral alterations induced by PCB exposure during development; this is mainly due to differences in the PCB mixtures used, as well as in the levels, length and time of exposure. Among the effects observed in male and female animals exposed even to low maternal doses are: alterations of estrous cycles and fecundability, decreased semen quality, impaired gonadal steroidogenesis, and morphological changes of the reproductive tract. Some epidemiological observations also indicate the induction of changes in human reproductive capacity such as alterations in menstrual cycles or in fecundability, as well as effects on semen quality and on sexual maturation (Negri-Cesi et al., 2007; Ulbrich and Stahlmann, 2004).

Recent experiments performed in our laboratory (Colciago et al., 2006), using a technical PCB mixture (Aroclor 1254), demonstrated that a short prenatal exposure, from gestational day (GD)15 to GD19, affects hypothalamic 5alpha-R2 expression in the GD20 females, but not in males. No data are presently available on the long-term effect of perinatal PCB exposure on the expression of aromatase and the 5alpha-Rs.

Therefore, we planned a multitasked study in which PCB liver and brain accumulation, along with the measurements of many morpho-functional parameters have been evaluated from birth to adulthood in the same groups of animals exposed to a reconstituted PCB mixture of four "indicator congeners" (PCB 126, 138, 153 and 180) commonly found in wild animals and human tissues (Bachour et al., 1998; Lanting et al., 1998; Ramos et al., 1997). PCB 138, 153 and 180 are known to interfere with hormonal steroid action, behaving as ER agonists/antagonists; PCB 138 behaves also as AR antagonist (Bonefeld-Jorgensen et al., 2001); PCB 126 exhibits a dioxin-like effect through AhR activation (Maier et al., 2007) and is known to affect the female reproductive system (Sakurada et al., 2007). The exposure covered the critical period of brain sex differentiation (from GD15 to weanling (PN21)). The first part of the study, which focuses on PCB effects on somatic growth, thyroid and growth hormone axes and bone mass has been reported in Cocchi et al. (2009). The aims of this second part are: a) to evaluate the expression pattern of aromatase, 5alpha-R1, 5alpha-R2 in fetuses (GD20), infant (PN12), weaning (PN21) and young adult (PN60) male and female animals exposed to PCBs during development; b) to correlate these parameters with the main landmarks of the reproductive system development (testicular descent, puberty onset, estrous cycle regularity) and copulatory behavior; c) to evaluate possible PCB-induced alterations of some non reproductive behaviors (locomotor activity, learning and memory); d) to assess depression and anxiety behaviors, since the two 5alpha-Rs are also involved in the formation of neurosteroids possessing anxiolytic/anesthetic properties.

Materials and methods

Animal care and PCB administration

Animal care and PCB treatment are described in details in the first part of this two section presentation (Cocchi et al., 2009). Briefly, time pregnant Sprague–Dawley rats (CD SPF/VAF, Charles Rivers, Calco, Italy) were individually housed in animal quarters in normal condition (14 h light–10 h dark; environmental temperature: 21–23 °C). They were fed a standard pellet diet and water was provided *ad libitum*.

In a first set of experiments, in which the enzyme expression pattern and reproductive parameters were evaluated, five dams/group/time and their litters were euthanized at GD20, PN12 and PN21. In these experiments, the mean n of animals/litter was 13 ± 2, not different between control and treated groups, therefore the litters were not culled at birth. After PN21, the remaining litters were separated by sex and treatment and housed (four rats/cage) with food *ad libitum*; food consumption did not differ between control and exposed animals. In all the studies, a maximum of 2 animals/sex belonging to the same litter were used. For the behavioral tests, a second set of adult male and female exposed rats, belonging to six (control) or seven (treated) litters, were utilized. Animals belonging to different litters (both males and females) were used for each test. The reconstituted PCB mixture was composed of the same amount of PCB 138, 153 and 180 (each representing one third of the total) and of PCB 126 at a concentration 10^4 times lower. Dams were treated subcutaneously from GD15 to GD19 with 10 mg/kg/day of the mixture, dissolved in 0.1 ml peanut oil and then left to deliver