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Effects of prenatal chlordecone on sexually differentiated behavior in adult rats

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Abstract

In rodents, exposure to estrogens during early development masculinizes the structure and function of the brain. The effects of early exposure to estrogens or estrogenic compounds can be evaluated by neurobehavioral testing after puberty. In this study, the effect of developmental exposure to the chlorinated pesticide, chlordecone (CD) on sexually differentiated behaviors in adults was investigated because CD binds to estrogen receptors and causes estrogenic effects in the reproductive tract of humans and rodents at relatively high doses. Pregnant Sprague–Dawley rats were exposed to 5 mg/kg CD by intraperitoneal injection on gestation day 16 (GD 16). Offspring were gonadectomized on postnatal day 50 (PN 50) to remove the effects of circulating hormones and were sequentially tested for sex-typic spontaneous behaviors in an open field and elevated plus maze, and for male and female mating behavior following the appropriate steroid regimen. Female rats exposed *in utero* to CD showed an increased ratio of inner to total crossings in the open field and significantly increased lordosis and male mounting as compared to female control rats. Male rats exposed *in utero* to CD showed significantly increased lordosis as compared to male control rats and no change in male mating behaviors. Permanent changes in adult behavior were consistent with both estrogenic and anti-estrogenic actions following developmental exposure to CD at the dose tested.

Keywords: Endocrine disrupting chemicals; Chlordecone; Sex behavior; Locomotion; Anxiety; Brain; Pesticides

1. Introduction

Humans and wildlife have been exposed to low levels of chemicals known as endocrine disrupting chemicals (EDCs), agents that disrupt endocrine pathways. However, the effects of EDCs on reproduction and development at environmentally relevant levels are still a matter of considerable controversy [13,64]. A good deal of attention has been given to agents that possess estrogen-like activity in animal experiments, including *in vitro* activity on estrogen receptors, and physiological activity in classic assays for estrogenic action such as the uterotrophic assay [6]. Some synthetic chemicals with reported estrogen-like activity include chlordecone, bisphenol A [45], alkylphenols [4,55], endosulfan, toxaphene, dieldrin, DDT [54], and methoxychlor [27].

The chlorinated pesticide chlordecone (CD) is a relatively strong estrogenic chemical *in vitro* compared to other environmental estrogens and CD acts as a competitive agonist for estrogen receptors (ER) α and β [40]. CD is associated with estrogenic effects in humans following cases of reproductive dysfunction and neurotoxicity in men exposed in an occupational incident in the 1970s [57,58]. For this reason it may be a model chemical to examine estrogen-like effects *in vivo*. In the uterotrophic assay, a dose of 50 mg/kg CD is as potent as the

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endogenous hormone 17β-estra diol (E_2) [29], and CD produces additive effects with E_2 in the uterus [33]. Additionally, pre- and postnatal exposure to high doses of CD leads to earlier vaginal opening, persistent vaginal estrus, and anovulation in adult female rats [23,24,50] and decreases cAMP levels [34] and increases progesterone receptors in the uterus of immature rats, but not adults [16]. The potential for CD to cause neurodevelopmental effects as a consequence of maternal exposures is plausible. CD is a lipophilic chemical, is readily absorbed into the digestive tract, and has a long half-life in blood [17]. CD is known to accumulate in maternal tissues, cross the placenta, and accumulate in fetal liver, brain, heart, and kidney [8,36].

In the brain, some studies suggest that CD activates estrogen receptors [26,31], while there is also evidence that CD does not mimic, but may attenuate, the action of E_2 [68]. Estrogen-like effects of CD in the brain include masculinization of sex behavior in adult female rats and hamsters following perinatal exposure [10,26] and progesterone receptor (PR) induction in the pituitary of rats [31]. Effects contrary to an ER-mediated mechanism include no induction by CD of PR in the brain [50], no induction of behavioral estrus in ovariectomized female rats [61], and no increase in the volume of the sexually dimorphic nucleus of the preoptic area (SDN-POA) in adult rats [10] after perinatal exposure. EDCs have been shown to have estrogenic effects on neurological development and the expression of sex-dependent behaviors in rats and mice [18–20,28,47,48,63].

Endogenous estrogens, synthesized from androgens by the enzyme aromatase, are involved in organizing both the brain and sexually differentiated behavior during development and activating sexual behavior in adult rodents [25,44,66]. Because the brain may respond to chemicals and may manifest changes at doses below where systemic toxicity occurs, it is an appropriate model for examining the effects of developmental exposure to EDCs. Behavior represents an integrated response of the nervous system that can reveal functional changes important to the overall fitness and survival of the individual. Adult male and female rodents display differences in both reproductive and non-reproductive behaviors including sex behaviors such as mounting and lordosis and non-sexual behaviors such as play, aggression, learning, activity, and response to novelty [3,5,11,14,35]. Many of these behaviors are sensitive to changes in estrogen-dependent signaling and EDCs during critical periods of pre- and postnatal development in rodents [7,9,12,15,19,20,26,27,30,37,39,46–48,51,53,56,58,62,63,65].

The purpose of this study was to test the hypothesis that prenatal exposure to CD acts through hormone-mediated organizational mechanisms in the central nervous system to alter hormone-activated behaviors in adult rats. To test this hypothesis, rats were exposed to CD *in utero* via maternal dosing during the sensitive organizational period for sexual differentiation of the brain. Offspring were then tested as adults for exploratory behavior, anxiety, and sexual behaviors following gonadectomy and standard hormone treatments to remove the effects of interanimal variability in circulating hormone levels. The considerable literature on the effects of estrogen manipulation on neurobehavioral outcomes and the results of this study indicate that CD has permanent effects on steroid-mediated behavior consistent with both estrogenic and anti-estrogenic mechanisms. The neurobehavioral effects occurred at a relatively low dose causing no effect on growth or survival.

2. Methods

2.1. Treatment of animals

Chlordecone (CD; Cambridge Isotope Labs) was dissolved in sesame oil (Sigma). Timed pregnant Sprague–Dawley rats (Charles River) were given a single intraperitoneal (ip) injection of 5 mg/kg CD or sesame oil vehicle on gestation day (GD) 16. This dose of CD was chosen from the literature and preliminary experiments to have no effect on growth or survival of dams or offspring. The timing of the dose was selected as one day prior to the beginning of the critical period for differentiation of sexual behavior in the rat [43]. Although only one dose of CD was administered, CD has a long half-life [17] which may have prolonged availability. After a single dose to pregnant dams, CD is elevated in the fetus for 24 h and declines slowly. CD is also detectable in the milk of lactating rats given CD [36]. Litters were culled on the day of birth to 5–7 of each sex per litter and some pups were removed from the litters on PN 2–12 for a separate experiment, but at all times the litter sizes and sex ratios were kept the same in exposed and non-exposed groups. On postnatal day (PN) 12, one male and one female rat pup from each of 10–12 control and 12 treated litters were fostered to a dam from the same treatment group, so that each litter contained 4 male and 3–4 female pups. Animals were weaned at PN 22 and housed separately by sex and treatment in groups of 2–3 animals/cage under a 12 h reversed light cycle (light off at 1100; light on at 2300). On PN 52–53, adult male and female rats were weighed and gonadectomized under ketamine (25 mg/kg) and acepromazine (75 mg/kg) anesthesia to remove the effects of interanimal variability in circulating hormone levels. Animals recovered from surgery for one week before behavioral testing began at PN 60. All procedures were approved by the University of Maryland, Baltimore Institutional Animal Care and Use Committee (IACUC).

2.2. Locomotor activity and anxiety

Gonadectomized offspring of treated and control dams (10–12 per group as described above) were tested for neurobehavioral function using two tests of ambulatory activity and response to novelty — an open field test, and an elevated plus maze test. The open field is a test frequently used to measure the response of an animal to an unfamiliar environment [56]. The elevated plus maze is a more specific test that measures aversion to the open arms of the maze and it has been used extensively to identify anxiolytic and anxiogenic drugs [35].

2.2.1. Testing in the open field

Each animal was tested on PN 60 between 3 and 6 h after lights out in a standard open field apparatus consisting of a