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ScienceDirect
Neurotoxicology and Teratology 28 (2006) 482–488
NEUROTOXICOLOGY AND TERATOLOGY
www.elsevier.com/locate/neutera

Learning deficits expressed as delayed extinction of a conditioned running response following perinatal exposure to vinclozolin

Susan M. André, Vincent P. Markowski *

Maine Center for Toxicology and Environmental Health, University of Southern Maine, Portland, Maine, 04104, United States

Received 7 November 2005; received in revised form 30 March 2006; accepted 3 April 2006
Available online 9 June 2006

Abstract

Vinclozolin (Vz) is one member of a group of fungicides whose metabolites are androgen receptor antagonists. These fungicides have been shown to block androgen-driven development and compromise reproductive function. The current study sought to determine if Vz also affects learning following exposure to low doses during the perinatal period. To test this, an androgen-dependent behavior was examined, the extinction of a previously reinforced running response. Pregnant Long–Evans rats were administered a daily oral dose of 0, 1.5, 3, 6 or 12 mg/kg Vz from the 14th day of gestation through postnatal day 3. After reaching adulthood, male and female offspring were trained to run through a short alleyway for food reinforcement. Acquisition of the response was not affected by Vz exposure. However, males required more trials than females for response extinction once food was no longer available in the apparatus. Males exposed to 6 or 12 mg/kg Vz failed to show any extinction by the end of the procedure, while the lowest dose of Vz appeared to facilitate extinction in both male and female offspring. These results demonstrate that endocrine disrupting antiandrogens can alter nervous system development in addition to the reproductive system.

Keywords: Vinclozolin; Antiandrogen; Perinatal exposure; Learning; Operant behavior; Rat

1. Introduction

Vinclozolin (Vz) is a dicarboximide fungicide that is applied as a foliar spray to numerous fruit and vegetable crops intended for human consumption [30,39,45,47]. Along with other dicarboximide residues, Vz has been found in grapes [7,43] and imported wines [42]. Additionally, Vz is applied to grass and ornamental plants, presenting a post-application risk to golfers playing on treated courses or to children playing on treated sod. Fungicides can enter surface water in turf runoff [20], potentially contaminating the drinking water sources used by more than half of the U.S. population [44]. Fungicides originating from agricultural runoff and antifouling paint have been detected in sea and river water at levels that meet or exceed the European Union limit for drinking water quality [31,40].

Vz is regarded as the prototype for a group of related compounds that are known as environmental antiandrogens [19,34,38]. Metabolites of Vz have been shown to bind competitively to the rat, monkey and human androgen receptor [27,26]. High doses of Vz impair the development of male reproductive organs and reduce fertility, but are also associated with maternal toxicity and increased mortality in male offspring. For instance, daily maternal doses of 50–200 mg/kg from the 14th day of gestation to the third postnatal day (GD14–PND3) reduced the anogenital distance and blocked the testosterone-mediated regression of nipples in male rat neonates. Hypospadias, penile hypoplasia, ectopic testes, and lower seminal vesicle and ventral prostate weights were seen in surviving adults [18,17,21,48]. Much lower maternal doses of 3–12 mg/kg increased nipple retention and reduced anogenital distance but caused only mild hypoplasic effects in reproductive organs [18,21].

The abnormalities produced by Vz exposure are not limited to the reproductive system. In animal studies that examine lower levels approximating human exposure, functional impairments including behavioral changes are predominant [13]. Because the

* Corresponding author. Department of Psychology, University of Southern Maine, 96 Falmouth Street, 178 Science Building, Portland, ME 04104-9300, United States. Tel.: +1 207 228 8174; fax: +1 207 228 8057.
E-mail address: markowski@usm.maine.edu (V.P. Markowski).

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doi:10.1016/j.ntt.2006.04.002

mammalian brain is masculinized by androgens during critical periods of development, environmental antiandrogens like Vz can target sex-differentiated behavior. For example, dietary exposure to Vz during development significantly reduced courtship behavior in juvenile male guppies [2]. When adults were exposed, doses at least an order of magnitude higher were required to produce similar effects [3]. In Japanese quail, a single exposure on day 4 of incubation disrupted the development of sexually dimorphic brain structures as well as male copulatory behavior [33]. In rats, low doses during the perinatal period significantly increased play behavior in juvenile males but not females [8]. On the other hand, higher doses limited to the neonatal period decreased play behaviors in male offspring [22,23].

The behavioral procedure used in the present study was based on work by Guillamon and others who examined the acquisition and extinction of a food-reinforced running response by rats in a relatively short, straight runway following endocrine manipulations during the neonatal period. This group found that control males and females resemble each other during the acquisition of the running response, but males extinguish more quickly after reinforcement is withdrawn. Females that are exposed to exogenous testosterone on PND1 or estradiol benzoate on PND1–8 have extinction functions that resemble control males. In contrast, males that are castrated on PND1 or are treated with the antiestrogen, tamoxifen, on PND1–8 resemble control females in adulthood [12,41]. These results strongly support the idea that gonadal steroids acting during a neonatal critical period are responsible for organizing the sex difference in extinction rates observed in adulthood. However, since the onset of this critical period in male rats is associated with a testosterone surge late in gestation [46], it is quite likely that an antiandrogen like Vz would function as an endocrine disrupter following perinatal exposure.

The current procedure and apparatus are similar to that used by Guillamon and others. However, this study examined litters of rats that were exposed during the perinatal period to low levels of Vz. We hypothesized that Vz would produce a dose-related demasculinization/feminization of the male offspring and decrease their rate of extinction of a conditioned running response. In a previous study, littermates of the current subjects produced significantly fewer total erections during tests of ex copula penile reflexes. In that study, all of the Vz exposure groups were affected, even at maternal doses as low as 1.5 mg/kg [8].

2. Methods

2.1. Breeding and exposure

Long–Evans hooded rats (Harlan, Indianapolis, IN) were allowed to acclimate to the University of Southern Maine Vivarium quarters for 2 weeks before breeding. All rats were fed standard pellet chow (Teklad Global 18% Protein Rodent Diet) ad libitum and were maintained on a 12-h light/12-h dark cycle in a barrier facility room with an ambient temperature of 68 ± 2 °F and 40–60% humidity.

For breeding, two females were housed with each stud male and vaginal smears were examined every morning for the presence of sperm. A sperm-positive smear was regarded as GD0. Pregnant rat dams were randomly assigned to one of the following exposure conditions: 0, 1.5, 3, 6 or 12 mg Vz/kg maternal bodyweight (Crescent Chemical Co. Inc., Islandia, NY). Vz was dissolved in corn oil and the appropriate volume (approximately 0.5–1.5 ml) was administered to the dams via gavage from GD14–PND3. Fresh solutions were prepared each day prior to dosing. Vz was not administered on the day of parturition (PND0).

Maternal body weights were recorded daily during the gestational period. Litter size, sex distribution, pup weights and anogenital distances were recorded on PND1 and every 4 days thereafter. Litters were culled to eight offspring on PND4, maintaining equivalent sex distributions when possible. After weaning on PND21, offspring were housed with same-sex littermates until PND60. After PND60, offspring were housed individually in order to maintain their bodyweights (males, 300–320 g; females, 200–220 g) with a daily feeding schedule. Training for the behavioral procedure began during the PND60–80 period. All animal procedures complied with approved institutional animal care protocols and in accordance with NIH guidelines [37]. Animal care and welfare was supervised by a veterinarian and an AALAS-certified Registered Laboratory Animal Technologist.

The breeding and exposure procedure yielded a total of 51 viable litters. From this cohort, 10, 7, 10, 9 and 7 male–female pairs of littermates from the 0, 1.5, 3, 6 and 12 mg/kg groups were assigned, respectively, to the conditioned running procedure. The remaining litters and littermates of the assigned animals were used to examine social play behavior and penile erections and are reported in Colbert et al. [8].

2.2. Apparatus

The apparatus consisted of a straight runway (9.5 cm wide × 12.5 high × 74 long; model E10-30SN, Coulbourn Instruments, Inc., Allentown, PA) that was connected on one end to a start box and to a goal box on the other end. The start box was a polycarbonate mouse shoebox cage (15 cm wide × 12.5 high × 26 long) with an access hole cut through one end. The goal box was a standard rat operant chamber (model H10-11R-TC, Coulbourn Instruments, Inc.) that was outfitted with a pellet feeder and a food hopper with a photocell sensor. Movement through the runway was controlled by automated guillotine doors at each end. The apparatus was controlled by a Habitest Universal Linc (model L91-04SHS, Coulbourn Instruments, Inc.) interfaced to a pc running the Graphic State (v1.01) programming system, which preserves behavioral events in real time.

2.3. Runway procedure

The behavioral test consisted of three phases. For the first day of the pretraining phase, naïve rats were individually allowed to explore the runway for 5 min. During days 2 and 3 of