Environmental Research 109 (2009) 400–404

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Effects of vinclozolin, an anti-androgen, on affiliative behavior in the Dark-eyed Junco, *Junco hyemalis* ☆, ☆☆

Danielle Satre, Michael Reichert [1], Cynthia Corbitt *

Department of Biology, University of Louisville, 139 Life Sciences, Louisville, KY 40292, USA

ARTICLE INFO

Article history:
Received 27 June 2008
Received in revised form
8 January 2009
Accepted 15 January 2009
Available online 3 March 2009

Keywords:
Songbird
Endocrine disruption
Female preference
Social behavior

ABSTRACT

Endocrine disrupting chemicals (EDCs) produce changes in physiology and behavior via diverse mechanisms including acting as hormone mimics or antagonists, affecting intracellular signaling pathways, and altering hormone production pathways. The fungicide vinclozolin acts as an anti-androgen and is known to affect affiliative behaviors in rodents, fish and amphibians. To investigate the possible effects of exposure to EDCs on reproductive behavior in a wild population of songbirds, we examined the effects of vinclozolin in wild-caught Dark-eyed Juncos (*Junco hyemalis*). For this and many other temperate songbird species, testosterone has powerful activational effects on affiliative behaviors in adulthood. We hypothesized that vinclozolin would affect male behaviors associated with female preference. Male juncos received daily oral gavage for 10 weeks with 2 mM vinclozolin in vehicle or vehicle alone. Juncos were photostimulated (16L:8D) to induce breeding behavior. Each pair of a treated and non-treated male was presented to an estrogen-primed female to assess female preference. Seven of eight females exhibited a strong preference for a male exposed to vinclozolin over a control male ($p = 0.01$). The only significant difference in measured male behaviors was increased beak wiping in controls ($p = 0.006$) and there was no difference in gonad size or brain weight ($p > 0.05$ for each). Our data suggest that estrogen-primed female juncos prefer to associate with male juncos exposed to this anti-androgen. This finding demonstrates that environmentally occurring anti-androgens can affect the social behavior of this species. To our knowledge, this is the first study to show that vinclozolin has effects on the social behavior of songbirds.

1. Introduction

Endocrine disrupting chemicals (EDCs) are found in the environment and can affect the physiology, morphology and behaviors governed by the endocrine system, particularly those regulated by sex steroids (André and Markowski, 2006; Bayley et al., 2002; Colbert et al., 2005; Edwards et al., 2006; Thibaut and Port, 2004). Subtle changes in physiology and behavior occur because EDCs act via various mechanisms including acting as hormone mimics or antagonists (reviewed by McLachlan, 2001; Damstra, et al., 2002). Evidence of effects of EDC exposure is accumulating, having been observed in several wildlife taxa including all vertebrate classes and some invertebrates. Recently it has been discovered that some EDCs can have transgenerational effects due to abnormal DNA methylation patterns (Anway et al., 2004; Chang et al., 2006; McLachlan and Burow, 2001; Price et al., 2007).

Vinclozolin (3-(3,5-dichlorophenyl)-5methyl-5 vinyl-oxazolidine-2,4-dione) is a dicarboximide fungicide used widely on plants, fruits, vegetables and turf sod. This chemical has been characterized as an androgen receptor antagonist *in vitro* and *in vivo* (Gray et al., 1999; Kelce et al., 1994, 1997; Monosson et al., 1999). Vinclozolin and its metabolites (M1 and M2) have the ability to bind to androgen receptors and prevent endogenous androgens from binding and eliciting a normal hormonal response (i.e., gene transcription). Additionally, this chemical and its metabolites have been shown *in vitro* to have antagonistic effects on the function of progesterone, glucocorticoid and mineralocorticoid receptors, as well as agonistic effects on the function of estrogen receptors (Molina-Molina et al., 2006), although *in vivo* effects are generally consistent with its anti-androgenic properties. Vinclozolin has been shown to have many adverse effects on reproductive structure and function in a variety of animals. For example, vinclozolin decreased secondary sex characteristics (Baatrup and Junge, 2001), spermatogenesis (Bayley et al., 2002)

☆ *Funding*: This work was funded by NSF Grant IBN-0137548 to Cynthia Corbitt.
☆☆ *IACUC*: This study was approved by the University of Louisville Institutional Animal Care and Use Committee, protocol ♯02042, prior to bird capture or use.
* Corresponding author. Fax: +1 502 852 0725.
E-mail addresses: msrgh9@mizzou.edu (M. Reichert), cynthia.corbitt@louisville.edu (C. Corbitt).
[1] Current address: 213 Tucker Hall, Division of Biological Sciences, University of Missouri, Columbia, MO 65211, USA.

0013-9351/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.envres.2009.01.004

and female fertility (Bayley et al., 2003) in the guppy, *Poecilia reticulata*. In Japanese quail, vinclozolin impaired male reproductive behavior (McGary et al., 2001). Developmental exposure to vinclozolin in male rabbits impaired follicle-stimulating hormone (FSH) secretion, spermiogenesis and sexual function (Veeramachaneni et al., 2006). Studies in rats demonstrate the ability of vinclozolin to alter many hormonally regulated behaviors including play behavior (Colbert et al., 2005; Hotchkiss et al., 2002), learning (André and Markowski, 2006), locomotor activity (Flynn et al., 2001) and reproductive behaviors (Colbert et al., 2005). Additionally, negative effects of vinclozolin on reproductive structures are well documented, as reviewed by Gray et al. (2005). Moreover, these effects on reproductive structure, function and attractiveness have been shown to be transgenerational, affecting male rats for up to 3 generations via epigenetic effects due to abnormal DNA methylation when administered to pregnant females during offspring gonadal differentiation (Anway and Skinner, 2006; Anway et al., 2005; Crews et al, 2007).

Most previous work on the effects of vinclozolin has been focused on developmental exposure. Few experiments have investigated the behavioral effects of vinclozolin exposure in adulthood. The model system used in this experiment is a temperate zone songbird, the Dark-eyed Junco (*Junco hyemalis*). Testosterone (T) in juncos and other temperate zone songbird species has powerful activational effects on social behavior in adulthood, making the Dark-eyed Junco an attractive model for EDC research involving effects of anti-androgen exposure in adulthood. Because these birds are photoperiodic, it is easy to manipulate their physiological state in the laboratory to resemble that of the breeding season and stimulate the hypothalamo-pituitary-gonadal (HPG) axis to increase sex steroid production. The effects of T during this time include mediation of social behaviors such as mating and aggression. In songbirds, T is known to affect a male's ability to obtain territories and attract mates (Catchpole and Slater, 1995).

To investigate the possible effects of male exposure to vinclozolin on female preference, we examined the effects of vinclozolin given to wild-caught, photostimulated male juncos on estrogen-primed female junco affiliative behavior. We hypothesized that vinclozolin would disrupt male behavior and negatively affect the ability of males to attract females.

2. Methods

2.1. Animal capture and care

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Louisville before being conducted. In addition, state and federal collection permits were obtained prior to animal capture. Dark-eyed Juncos were caught using millet-baited mist nets near Louisville, Kentucky (December–February). Upon capture, plumage color and wing chord length were used to determine whether birds were male or female (Pyle, 1997); blood samples were taken and initial body condition and health were assessed. Birds were transferred to the lab and housed in individual cages (21 cm × 28 cm × 38 cm) throughout the experiment, with males and females in separate rooms. Ambient temperature was maintained at 25 °C. Birds were exposed to fluorescent lighting on short days (8L:16D).

All experimental subjects were fed a mix of millet and a phytoestrogen-reduced (0.03 μg/g) commercial diet (made by Purina with alcohol-washed soy protein provided by Central Soya, now The Solae Company, St. Louis) until gradually transitioned to the Purina diet only, which they were given for the remainder of the experiment *ad libitum*.

2.2. Treatments

Sixteen individual males were randomly placed into either a control group or a treatment group. In addition, males were paired, 1 from each group, based on age, plumage color and wing chord length. All males were dosed daily for 10 weeks via oral gavage beginning March 3. Control birds received 100 μL vehicle (organic canola oil), while treated birds received 100 μL of 2 mM vinclozolin (Chem Service, West Chester, PA) dissolved in vehicle (2.86 mg/kg day). This dose was chosen based on data from a previous study that investigated effects of 3 doses of vinclozolin (0.2, 2.0 and 20 mM) and 100 μg/bird flutamide (unpublished data). In the previous study, effects of the 2.0 mM concentration of vinclozolin had effects similar to those of flutamide. Unfortunately, not enough birds could be captured for this study to include a flutamide group. Seven days after dosing began, males and females were photostimulated and remained on long days (LDs, 16L:8D) for the remainder of the experiment to simulate the breeding season.

Blood was obtained weekly from the brachial vein. Samples were taken immediately upon removal of the bird from its cage. Blood samples were centrifuged and plasma was collected. Plasma was then stored at −20 °C until analyzed for testosterone concentration by enzyme immunoassay (EIA). However, the intra-assay coefficients of variance (CV) were unacceptably high (67 out of 142 samples had a CV of more than 20%); so the hormone data are deemed too unreliable to be presented here. Unfortunately, the blood samples were not large enough to perform another assay.

Subsequent to blood sampling, the width of the cloacal protuberance (CP) was measured using dial calipers (±0.1 mm); fat score was assessed on a scale of 0–5, taking into account both furcular and abdominal fat; and body mass was measured using a tared digital scale (±0.1 g). Males also underwent laparotomy at week 4 (i.e., after 3 weeks of LD) to measure gonad size.

At the end of the experiment, birds were anesthetized with ketamine/xylazine and sacrificed by intracardiac perfusion with 4% paraformaldehyde. Gonads were removed, patted dry and measured (weight and length).

2.3. Behavioral tests

Preference tests were conducted starting on day 28 of the long day cycle, 1 week after males began singing. A modified preference apparatus was used, which was based on the standard Y-shaped arena (Fig. 1) used in previous mate choice experiments (Enstrom et al., 1997; McGlothlin et al., 2004). Females were primed with one 10 mm silastic capsule containing 17β-estradiol 3 weeks prior to testing. Twenty-four hours before each preference trial, 1 female was randomly selected and allowed to inspect the choice apparatus for 1 h (20 min in her home

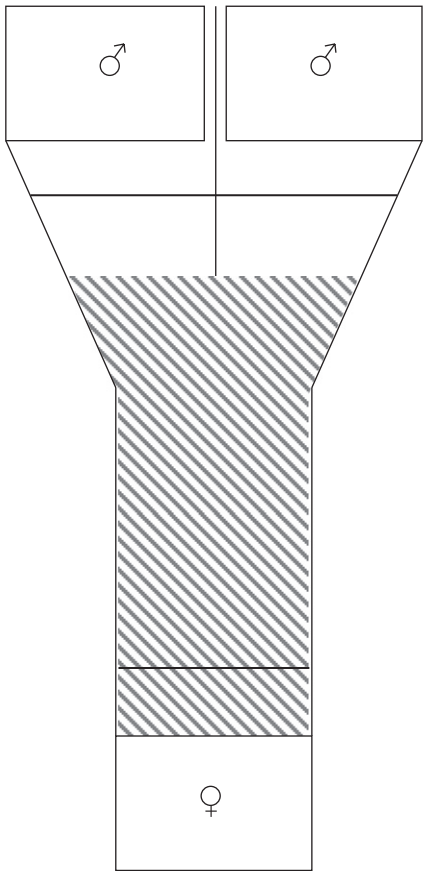

Fig. 1. Preference apparatus. The white areas depict choice areas, while the shaded area depicts non-choice area. The thin black lines in front of the cages represent perches available to the focal female.