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Assessing the consequences of the pesticide methoxychlor: neuroendocrine and behavioral measures as indicators of biological impact of an estrogenic environmental chemical

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Abstract

Japanese quail provide an advantageous avian model for assessing long-term biological consequences of endocrine disrupting chemicals (EDCs). These studies examined route of exposure and vulnerability to biological impact of EDCs over the life cycle in a precocial avian model, the Japanese quail. Embryonic exposure occurs with maternal deposition and methoxychlor (MXC) accumulated with maternal exposure. Egg injections of MXC or estradiol at selected stages of development impacted hypothalamic neuroendocrine systems in hatchlings and affected sexual maturation, with evidence for long-term effects on neurotransmitters and male behavior. Two-generation dietary studies were conducted to examine transgenerational effects of EDCs. Adult quail (P1) were exposed to dietary MXC (0, 0.5 and 5 ppm), with continued exposure in their offspring (F1), and control diet for all F2 chicks. Toxicological end points, including fertility, hatching success, and 14-day viability were unaffected. F1 and F2 male offspring from MXC-treated pairs MXC had impaired mating behavior and altered plasma hormones. These studies confirm neuroendocrine and behavioral measures as reliable indices of exposure to an estrogenic EDC. Moreover, maternal deposition remains a primary route of EDC exposure, with potential deleterious consequences for field birds, especially precocial species that appear to be particularly sensitive to embryonic EDC exposure.

Keywords: Neuroendocrine systems; Sexual behavior; Estrogens; Japanese quail

1. Introduction

Endocrine disrupting chemicals (EDCs) include a number of environmental chemicals and chemicals found in plant products that interact with an endocrine system, often due to activity as a hormonal mimic [43]. Unfortunately, it was not apparent until relatively recently that pesticides, herbicides, industrial chemicals and even plant hormones have endocrine activity in vertebrate species. Recognition of the potential impact that these compounds may have on wild bird populations has stimulated research to characterize the effects of these chemicals and consideration of phylogenetic variation in biological response to exposure (for review, see [30]). Once reliable end points have been identified and characterized, this will permit non-invasive assessment of EDCs in the environment [14,15].

Given our current understanding of the activity of EDCs, many of the traditional measures for toxicity of compounds appear to be relatively insensitive in birds as well as in other species. These measures have included overall health and food intake, fertility, viability of offspring, gross morphology of gonads and general measures of growth and development. Current toxicity testing has not included endocrine specific

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variables such as plasma steroid hormones, reproductive neuroendocrine or behavioral end-points. It is critical to establish testing methods that utilize meaningful variables, and quantitatively consider relative change in these end-points, which are responsive to EDCs and reliable indicators of biological impact. In total, the test methods for EDCs should include overall measures of reproduction, such as fertility, viability and number of chicks etc., as well as additional end-points indicative of development and function of the hypothalamic pituitary gonadal (HPG) axis. These additional measures will provide sensitive and reliable indices of the potential for a compound to have endocrine disrupting activity.

The critical role of steroid hormones has been demonstrated in embryonic development and sexual differentiation as well as during sexual maturation, with many studies focused on the neural mechanisms involved in modulation of endocrine and behavioral components of reproduction [3,7,12,20,25–27,45]. Administration of exogenous gonadal steroids altered sexual differentiation, resulting in sex differences in later male or female behavior [2,3,7,31]. In quail, the timing of embryonic exposure to testosterone, E2, fadrozole or tamoxifen by embryonic day (E12) altered later expression of sexual behavior; defeminized females [7,30,32,33]. In addition, early steroid exposure also alters gonadal development, with fadrozole and tamoxifen exposure producing defeminization of the ovary and accessory structures [9,12,16,32]. Finally, female quail given E2 implants were found to transfer estradiol to offspring via the yolk [4]. This provides further evidence that maternal deposition of lipophilic hormones or EDCs are a likely pathway for exposure in field species.

Methoxychlor (MXC) is a widely used pesticide that gained in popularity after the ban on DDT in 1972. In rats, MXC clearly impacts both male and female embryos [6,13,17,41]. There is also evidence for imprinting leading to altered adult sensitivity to EDCs [35]. Furthermore, it has been demonstrated that while MXC itself may be short-lived, its metabolites are also biologically active and these compounds may imprint embryonic tissues, resulting in altered responses in adults [5,6]. Therefore, MXC is considered a pesticide with the capacity to induce endocrine disruption, in the form of the parent compound as well as its metabolites.

Because of its wide usage, there is a great deal of potential for exposure to MXC in the field for birds and impact at the individual and population levels. This pesticide has long been considered safe for use because of its short half-life; however, most of these early tests on MXC were conducted on adult or near adult birds. Given the estrogenic nature of MXC, it is reasonable to suspect that even short-term maternal exposure could transfer to the egg/embryo and have long-term effects. A bioassay has been developed in the zebra finch that will prove useful to assess specific responses in passerine [20]. Studies in chickens and Japanese quail have shown impact of EDC exposure during embryonic and adult phases of the life cycle [9,13,16,18,23,28,29,31,37,38]. We will discuss our findings in the Japanese quail in more detail below, concentrating on the estrogenic pesticide, MXC, and its positive control, 17-β estradiol.

2. The Japanese quail model

Previous studies have shown that the male Japanese quail is exquisitely sensitive to the effects of exogenous estradiol especially during critical periods of sexual differentiation of neural systems. In addition, the hormonal basis for sexual differentiation in avian species differs from mammals, thereby making mammalian tests potentially inadequate for assessing EDC impact on avian species. Regulatory agencies have routinely used avian species indigenous to North America for testing toxic chemicals for industrial and agricultural applications. These species have included mallard ducks and northern bobwhite quail. However, there are relatively few data available on the HPG axis in these species. Conversely, a great deal is known about neuroendocrine regulation of reproduction in Japanese quail, but few data have been collected on effects of EDCs on neuroendocrine systems in quail. Therefore, our research has focused on investigating the impact of EDCs in the Japanese quail as a model for precocial avian species and determining end-points that provide reliable and sensitive indices of endocrine disruption.

The avian model has a distinct advantage over mammalian models of embryonic neuroendocrine differentiation. The maternal contribution to the egg is limited to the period between formation and oviposition [9,10,39]. Researchers can thereafter manipulate embryo conditions in a controlled environment to better understand development. It thus provides a convenient model in which to study the effects of chemical exposure to the embryo.

Further, a great deal of information is available about the role of steroid hormones in embryonic development and maturation of the Japanese quail, and the mechanisms involved in these processes [3,7,21,22,25–27]. Measurement of endogenous steroids during embryonic development has shown that both adrenal and gonadal steroids contribute to circulating hormone levels. In the female embryo, plasma E2 rose until hatch and decreased, posthatch [1,24,29]. Increased 5β reductase enzyme activity was found in the brain of male quail embryos between E7 and E15, possibly acting to protect males from being behaviorally demasculinized by inactivating testosterone [8]. In males, plasma androgen peaked at E14–E17, and declined post-hatch [24]. A similar embryonic pattern in plasma gonadal steroids, including an adrenal contribution has also been documented in the domestic chicken [21,36]. Females treated with estradiol at E10 or E12 had higher preoptic dopamine (DA) content as adults and treated males had relatively lower preoptic norepinephrine (NE) content and were behaviorally demasculinized. The preoptic-lateral septal region (POA-SL) is also an important area because it contains many of the gonadotropin releasing hormone-I (GnRH-I) cell bodies and regulates copulatory behavior, while the median eminence (ME) is the site of GnRH-I release. Finally, neurotransmitters, including