

Inter-species variation in yolk steroid levels and a cowbird-host comparison

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Hahn, D. C., Hatfield, J. S., Abdelnabi, M. A., Wu J. M., Igl, L. D. and Ottinger, M. A. 2005. Inter-species variation in yolk steroid levels and a cowbird-host comparison. – J. Avian Biol. 36: 40–46.

We examined variability in yolk hormone levels among songbird species and the role of yolk steroids as a mechanism for enhanced exploitation of hosts by the parasitic brown-headed cowbird *Molothrus ater*. Within-clutch variation in yolk steroids has been found in several avian species in single species studies, but few comparisons have been made among species. We found a large range of differences in yolk testosterone among the seven passerine species examined, with significant differences between those at the high end (song sparrow *Melospiza melodia*, red-winged blackbird *Agelaius phoeniceus*, and house sparrow, *Passer domesticus*) and those at the low end (eastern phoebe *Sayornis phoebe*, and house finch *Carpodacus mexicanus*). We also found that the testosterone level in cowbird eggs was intermediate in relation to host species levels and was significantly lower than that in three common cowbird hosts (song sparrow, red-winged blackbird, and house sparrow), but not significantly different from three others. Geographical comparisons of yolk testosterone levels in all cowbird subspecies and populations from several regions showed no significant differences, though a trend that deserves further exploration was the pattern of lowest level in the ancestral population of cowbirds in the central prairies and of highest level in the northwestern population where range invasion occurred approximately 40 years ago. The levels of 17 beta-estradiol were similar in the seven songbird species examined, which is consistent with current hypotheses that this hormone plays a role in embryonic sexual differentiation. Further investigation is needed to determine whether the large differences observed among species in absolute level of yolk testosterone are the relevant focal point or whether target tissue sensitivity differences mediate the effects of this yolk steroid, particularly between parasitic and non-parasitic species.

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Introduction: neurobiological impact of environmental estrogens

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Abstract

This paper provides an introduction to a special issue dedicated to the action of environmental estrogens on neural circuits and behavior. The problem of endocrine disrupting chemicals (EDCs), i.e. chemicals that have the capacity to interfere with the endocrine system, has gained increasing attention as it has become clear that these environmental contaminants may be active in humans, as well as in wildlife and domestic animal species. The majority of the early investigations were aimed at the discovery of the toxicological effects of the EDCs, but biomedical observations were among some of the first indications that estrogenic compounds may exert deleterious effects, even some time after exposure. The data derived from women exposed prenatally to diethylstilbesterol provided powerful evidence for long-term effects and endocrine disruption associated with selected compounds. The examination of wild animal populations exposed to industrial chemicals showed that the chemical exposure, though nonlethal, left the individual impaired or even incapable of reproducing.

Among the multiple targets of the action of EDCs, several researches performed in recent years have investigated subtle modifications of the animal behaviors (reproductive, aggressive) that are likely to be related to alterations of specific neural pathways. We have, therefore, focused here on the behavioral studies as one of the more powerful tools to investigate EDCs effects on specific neural circuits.

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Keywords: Endocrine disruptors; Industrial chemicals; Xenoestrogens; Central nervous system; Brain circuitries; Behavior

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Effects of Dose and Glycosylation on the Transfer of Genistein into the Eggs of the Japanese Quail (*Coturnix japonica*)

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Soy isoflavones have been associated with several beneficial effects of soy in human diets. However, most soy is consumed by livestock in the Western countries. It is possible that isoflavones could be transferred and/or accumulated into animal products, which could become additional sources of dietary isoflavones for humans. Our objectives were to determine whether dietary isoflavone genistein could be transferred and/or accumulated into the eggs of Japanese quail (*Coturnix japonica*) and how the supplementation dosage and glycosylation of the isoflavone would affect this transfer. Adult reproductive female Japanese quail were randomly assigned to treatment groups that received encapsulated 50 or 100 mg genistein or 80 mg genistin per day (four quail per treatment) for 5 days. A control group (two quail) received placebo capsules. Eggs were collected prior to treatment and then daily for 15 days. The egg, separated into yolk and white, and pulverized quail diet were extracted in 80% methanol for 2 h and either centrifuged or filtered before evaporation of the solvent. The extracts were redissolved in 16% acetonitrile for high-performance liquid chromatography (HPLC) analyses. Genistein and genistein metabolites were detected in the egg yolks of treated quail. Trace concentrations of genistein were detected in the control group, due to the presence of genistein derivatives in the diet. Neither genistein nor its metabolites were found in egg white. Levels of genistein in the eggs increased significantly from the 3rd day of supplementation and reached the maximum about 2 days after the supplementation stopped. The higher dose of genistein supplementation resulted in higher genistein concentrations in egg yolks. Glycosylation decreased the transfer and accumulation of genistein into the egg yolks.

KEYWORDS: Genistein; genistin; egg yolk; Japanese quail; transfer; accumulation

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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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Available online 18 December 2004

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.

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

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Consequences of endocrine disrupting chemicals on reproductive endocrine function in birds: Establishing reliable end points of exposure

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Abstract

It has been difficult to establish reliable indices of exposure to endocrine disrupting chemicals (EDCs) appropriate for a variety of avian species because of a vast array of reproductive strategies. Data from mammals, reptiles and fish provide insight on likely mechanisms of action for EDCs. However, many of the effects of EDCs are weaker than the actions of the native hormones, making it difficult to assess adverse effects in domestic and wild birds. It is clear that differential sensitivity to EDCs exists across species, due to the timing and mode of exposure, compound toxicity and age of the individual. Our studies on EDCs are conducted in the quail model system, with focus on reproductive endocrine, neuroendocrine and behavioral responses. Studies have included EDC exposure, either by egg injection or via diet. Results from egg injection studies showed the following: (1) estradiol administered by embryonic day 12 demasculinized male sexual behavior, altered hypothalamic neurotransmitters and reduced hen day production and fertility in a dose dependent fashion, (2) methoxychlor (MXC) or vinclozolin impaired male sexual behavior in adult quail and (3) DDE exposure impaired reproductive and immune related end points. Two-generation studies were conducted on Japanese and northern bobwhite quail with dietary methoxychlor (MXC) exposure (0, 5 and 10 ppm) beginning in adults (P1), continuing in their offspring (F1), with F2 offspring raised on control diet. MXC exposure impaired male sexual behavior, hypothalamic catecholamines and plasma steroid hormones. Moreover, MXC exposure had reproductive consequences observable at both the lower and higher doses of MXC in

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F1 and F2 generations. These data demonstrate that embryonic EDC exposure interferes with sexual differentiation of neural systems that direct reproduction.

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Keywords: Endocrine disrupting chemicals; Neuroendocrine measures; Reproductive end points; Immunotoxicology

Reproductive Consequences of EDCs in Birds: What Do Laboratory Effects Mean in Field Species?

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Running title: reproduction and EDCs

Key words: avian model, endocrine disrupting chemicals, end points, biomarkers

Supported in part by U.S. EPA Grant R826134 and NSF #IBN 9817024 (MAO)

Abstract

The varied reproductive strategies of birds present a challenge in developing reliable indices for the assessment of effects of endocrine disrupting chemicals (EDCs). Precocial species, such as quail, appear to be most sensitive to EDC effects during embryonic development. Although the Japanese quail (*Coturnix japonica*) is a non-native lab species, its reproductive strategy is similar to that of many free-ranging species. Because a great deal is known about the reproductive biology of this species and Japanese quail have a short generation time, this species is an ideal candidate for testing EDC effects. In this review, we present data collected in a two-generation design with embryonic exposure to estradiol benzoate. This study was conducted to provide fundamental information for establishing reliable reproductive endpoints associated with estrogenic EDC exposure. Data were collected for a variety of endpoints, which were chosen as measures of reproductive capability and success. These reproductive fitness measures included fertility, hatching success, and offspring viability. Endocrine measures consisted of plasma hormone levels and gonad weight/condition. Neuroendocrine systems, such as the monoamine neurotransmitter systems regulate hypothalamic gonadotropin releasing hormone (GnRH) and reproductive behavior. Therefore, these variables would potentially be very sensitive indicators. Behavioral measures included reproductive behavior. Results showed that embryonic estradiol exposure affected endocrine and behavioral responses in males and impacted productivity in females. Therefore, quail provide an excellent model to determine fundamental actions of EDCs. The laboratory trials then serve as a basis for the extrapolation of findings of controlled laboratory studies to effects that may be observable in free-ranging species.