Role of steroid hormones in Trichinella spiralis infection among voles

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Role of steroid hormones in Trichinella spiralis infection among voles. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1362–R1367, 1999.—Males are generally more susceptible to parasite infection than females. This sex difference may reflect the suppressive effects of testosterone and enhancing effects of estradiol on immune function. This study characterized the role of circulating steroid hormones in sex differences after infection with the nematode Trichinella spiralis. Because testosterone suppresses immune function and because polygynous males have higher circulating testosterone concentrations than monogamous males, sex differences in parasite burden were hypothesized to be exaggerated among polygynous meadow voles compared with monogamous prairie voles. As predicted, sex differences in response to T. spiralis infection were increased among meadow voles; males had higher worm numbers than females. Male and female prairie voles had equivalent parasite burden. Overall, prairie voles had higher worm numbers than meadow voles. Contrary to our initial prediction, differences in circulating estradiol concentrations in females, testosterone concentrations in males, and corticosterone concentrations in both sexes were not related to the observed variation in T. spiralis infection. Taken together, these data suggest that not all sex differences in parasite infection are mediated by circulating steroid hormones and that adaptive-functional explanations may provide new insight into the causes of variation in parasite infection.

FIELD STUDIES in both birds and mammals suggest that parasite prevalence and intensity (i.e., both the number of infected individuals and the degree of infection within an individual) are higher among males than females (25, 38). Although these data are suggestive, several factors, including exposure rates, social behavior, habitat, and diet, were not held constant and could contribute to the observed differences in parasite infection. However, studies in mice and rats have demonstrated that, even in a “controlled” laboratory setting, males are more susceptible to parasite infection than females, and this difference is related to the effects of sex steroid hormones on immune function (1, 2, 30, 35). In other words, sex differences in parasite burden reflect the suppressive effects of testosterone and enhancing effects of estradiol on the immune system. Experimental studies, mainly using laboratory rats and mice, have established that sex differences in parasite burden are reversed when males are gonadectomized and females are chronically injected with testosterone propionate (19, 35, 38).

The purpose of this study was to characterize the role of circulating steroid hormones in sex differences in two Microtus species after infection with the nematode Trichinella spiralis. This nematode was chosen because 1) T. spiralis is a parasite that can infect most rodents and has been identified in wild populations of Microtus species (34); 2) this parasite is not easily transmissible between individual rodents; and 3) T. spiralis has a direct life cycle and, therefore, does not require maintenance in an intermediate host (3).

This study also examined the hypothesis that sex differences in parasite burden reflect the mating system; i.e., sex differences should be exaggerated among polygynous compared with monogamous species (37). This controversial hypothesis has not been tested explicitly but represents an alternative to the traditional analyses of sex differences in disease prevalence that rely heavily on mechanistic explanations (9, 21, 32). Microtus species were chosen for this comparison because both monogamous and polygynous species have been characterized in this genus. Morphological, physiological, and behavioral data from field and laboratory studies rate prairie voles (Microtus ochrogaster) as one of the most monogamous species and meadow voles (M. pennsylvanicus) as the most polygynous Microtus species (4). Despite representing extremes of social organization for rodents, these Microtus species are very similar in terms of overall life history strategies, habitat use, and gross morphology (5). Although examining only two species has limitations (23, 29), comparisons of two congeneric species have been used successfully to address evolutionary principles about the role of the mating system in behavior, morphology, and physiology (8, 12, 13, 22). Thus, if sex differences in parasite infection are exaggerated among polygynous species, then sex differences in parasite infection should be more pronounced among meadow voles than prairie voles, and differences in sex hormone concentrations should be related to variation in parasite infection.

METHODS

Animals

Adult (>60 days of age) male (n = 10/species) and female (n = 10/species) meadow voles (M. pennsylvanicus) and

Klein, Sabra L., H. Ray Gamble, and Randy J. Nelson. Role of steroid hormones in Trichinella spiralis infection among voles. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1362–R1367, 1999.—Males are generally more susceptible to parasite infection than females. This sex difference may reflect the suppressive effects of testosterone and enhancing effects of estradiol on immune function. This study characterized the role of circulating steroid hormones in sex differences after infection with the nematode Trichinella spiralis. Because testosterone suppresses immune function and because polygynous males have higher circulating testosterone concentrations than monogamous males, sex differences in parasite burden were hypothesized to be exaggerated among polygynous meadow voles compared with monogamous prairie voles. As predicted, sex differences in response to T. spiralis infection were increased among meadow voles; males had higher worm numbers than females. Male and female prairie voles had equivalent parasite burden. Overall, prairie voles had higher worm numbers than meadow voles. Contrary to our initial prediction, differences in circulating estradiol concentrations in females, testosterone concentrations in males, and corticosterone concentrations in both sexes were not related to the observed variation in T. spiralis infection. Taken together, these data suggest that not all sex differences in parasite infection are mediated by circulating steroid hormones and that adaptive-functional explanations may provide new insight into the causes of variation in parasite infection.

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METHODS

Animals

Adult (>60 days of age) male (n = 10/species) and female (n = 10/species) meadow voles (M. pennsylvanicus) and
prairie voles (M. ochrogaster) were obtained from our breeding colony (16:8-h light-dark cycle, lights on 0600 Eastern Standard Time). Voles were maintained individually from weaning (21 days of age) in polycarbonate cages (28 × 7.5 × 13 cm) at 21 ± 2°C to avoid effects of social interactions on immune function (15–17). Food and tap water were available ad libitum. Sentinel animals were housed in the colony rooms and were screened regularly for the presence of common rodent diseases arising from parasitic, viral, bacterial, and fungal origins. Serological assessment revealed that all sentinel animals tested negative for the presence of any infection, indicating that our vole colonies are devoid of any existing disease. All procedures described below were approved by The Johns Hopkins University Animal Care and Use Committee.

Procedure

All animals were inoculated orally with 100 larvae of T. spiralis suspended in 0.85% sterile saline. Although this is the first experimental assessment of T. spiralis infection in voles, the dose used produces subclinical infection in laboratory rats and mice (3). After inoculation, animals remained undisturbed, other than routine cage cleaning, in their home cages for 30 days. Thirty days postinoculation is characterized as the chronic phase of infection, during which animals harbor infective, intramural, larval cysts of T. spiralis with the highest concentrations in the diaphragm (3). Furthermore, most studies characterizing physiological and behavioral effects of T. spiralis infection report pronounced effects during the chronic phase of infection (i.e., 30–40 days postinoculation; see Refs. 6, 11, 19, 26, and 27).

Thirty days postinoculation, all animals were lightly anesthetized with methoxyflurane vapors (Metofane; Schering Plough, Union, NJ) and were bled from the retroorbital sinus into heparinized tubes (50 µl/tube). The blood sampling procedure lasts <1.5 min. Blood samples were stored at −80°C and were used for analysis of plasma testosterone in males, estradiol in females, and corticosterone in both sexes using RIA. After bleeding, animals were killed by CO2 asphyxiation and cervical dislocation and were skinned and eviscerated, and muscle tissue was digested (using the procedure described below). The number of recovered larvae was counted for each vole using the appropriate dilutions.

T. spiralis digestion. Digestion of muscle tissue in an acidified pepsin solution releases live trichinae from cysts that develop in muscle tissue (7). Muscle tissue from skinned, eviscerated vole carcasses was ground to expedite digestion. Muscle samples were then digested in artificial gastric fluid containing 1% (wt/vol) pepsin and 1% (wt/vol) hydrochloric acid. Ground muscle tissue from each animal was added to the artificial gastric fluid (prewarmed to 37°C) and was stirred on a magnetic stirrer for 3–4 h at 37°C. The mixture was then allowed to settle for 15–20 min, and the upper two-thirds of the mixture was decanted. The remaining fluid with sediment was allowed to settle further for 15–20 min, after which the supernatant was aspirated. The remaining sediment was washed with tap water (37°C) and was allowed to settle for an additional 15–20 min. This washing step was repeated until the supernatant was clear. The remaining washed sediment was transferred to a 50-ml conical tube, allowed to settle, and aspirated down to a final volume of 10 ml. The sediment was then poured in a petri dish, and Trichinella larvae were counted using a dissecting microscope. Dilutions were made as necessary to facilitate counting.

Steroid hormone RIA. Plasma estradiol concentrations in females and testosterone concentrations in males were assayed by RIA using 125I kits purchased from ICN Biochemi-
cals (Carson, CA). The 17β-estradiol assay is highly specific; cross-reaction with other steroid hormones is <0.1%. The estradiol values were determined in a single RIA, and the intra-assay coefficient of variation was 5.3%. The testosterone assay is also highly specific; cross-reaction with other steroids is <0.1%. Testosterone values were also determined in a single RIA, with a 4.4% coefficient of variation. Corticosterone was measured using an 125I RIA kit (ICN Biochemicals) that had previously been validated for use in voles (33). The only deviation from the manufacturer’s protocol was the dilution factor for the plasma. The plasma samples in both species were diluted 1:2,121 in assay buffer (33). All plasma samples were assayed at the same time, and the intra-assay coefficient of variation was 4.8%.

Statistical Analyses

Because the worm burden data were not normally distributed, numbers of recovered T. spiralis larvae from each animal were log transformed and analyzed using an ANOVA with two between-subjects variables (species and sex). Sex differences in parasite infection were hypothesized, a priori, to be present in meadow voles only; thus, sex differences were examined within each species using independent one-tailed t-tests. If animals died before the end of the study, then their data were not included in the analyses; there were no species differences in mortality (1 or 2 animals per sex per species). Plasma testosterone concentrations in males and estradiol concentrations in females were not normally distributed and were analyzed using Mann-Whitney rank sum tests. Plasma corticosterone concentrations and body mass were assessed using ANOVAs with two between-subjects variables (species and sex). The combined contribution of steroid hormones to variation in parasite infection was investigated using multiple linear regression. Because all animals did not have enough plasma to assess steroid hormone concentrations, sample sizes vary from 8 to 10 animals per sex per species. All mean differences were considered statistically significant at P < 0.05.

RESULTS

T. Spiralis Larvae

When species were examined together, females had lower worm burdens than conspecific males [F(1,31) = 4.455, P < 0.05]. However, sex differences in parasite burden were only significant among meadow voles; fewer numbers of larvae were recovered from females than males [t = 1.995, degrees of freedom (df) = 15, P < 0.05; Fig. 1A]. Overall, prairie voles had greater numbers of larvae in muscle tissue than meadow voles [F(1,31) = 15.285, P < 0.05; Fig. 1].

Steroid Hormone Concentrations

Prairie voles had higher corticosterone concentrations than meadow voles [F(1,33) = 5.601, P < 0.05; Fig. 2]. Sex differences in corticosterone concentrations were apparent only among meadow voles; females had higher corticosterone concentrations than males [t = −2.319, df = 16, P < 0.05; Fig. 2].

Male meadow voles had higher testosterone concentrations than prairie voles (U = 113, P < 0.05; Fig. 3A). Female prairie voles had higher estradiol concentrations than female meadow voles (U = 59, P < 0.05; Fig. 3B). Plasma testosterone concentrations in males and
estradiol concentrations in females were not related to the number of recovered larvae from muscle tissue ($P = 0.15$ and $0.18$, respectively). Additionally, a multiple linear regression revealed that differences in steroid hormone concentrations did not account for variation between species or sexes in parasite infection ($P = 0.35$).

**Body Mass**

Sex differences in body mass were only apparent among meadow voles; males ($53.21 \pm 2.64$ g) weighed more than females ($36.92 \pm 2.64$ g; $F(1, 33) = 8.569$, $P < 0.01$). Prairie vole males ($42.48 \pm 2.07$ g) and females ($41.47 \pm 1.84$ g) had equivalent body mass. Body mass was not related to the number of larvae recovered ($P = 0.16$).

**DISCUSSION**

Males may be more susceptible to infection than females because of proximate/mechanistic factors, including differences in hormone concentrations or exposure to stressors, as well as evolutionary factors, such as differences in selection pressures between the sexes (37, 38). In the present study, females had lower worm burdens than conspecific males, and this sex difference was exaggerated among polygynous meadow voles. Male meadow voles had five times the number of larvae as conspecific females. Similar findings have been reported in hooded rats; males had three times as many T. spiralis larvae 30 days postinoculation compared with females (20). This sex difference was reversed if rats were gonadectomized as adults and females were treated with testosterone and males with stilbestrol for 14 days after inoculation, suggesting that sex steroid hormones influence sex differences in T. spiralis infection (20).

From a proximate perspective, high circulating testosterone concentrations may account for the increased number of T. spiralis larvae recovered from male compared with female meadow voles. Conversely, if circulating testosterone is immunosuppressive, then male prairie voles, which had lower testosterone concentrations than male meadow voles, should exhibit reduced parasite burden. In the present study, prairie vole males had lower testosterone values and harbored higher numbers of parasites than meadow vole males. Additionally, differences in testosterone concentrations among males were not related to variation in T. spiralis infection in these species. In the present study, sex steroid hormone concentrations were assessed at a single time point during the chronic phase of infection (i.e., 30 days postinoculation). Previous data in hooded rats suggest that hormonal manipulation (i.e., castration or hormone replacement) at the onset of infection alters T. spiralis burden 30 days later (19). Thus sex steroid hormonal concentrations at the onset of infection...
tion, or even during earlier phases of infection, may affect subsequent worm burden.

The testosterone values reported for infected males in this study do not differ from values previously reported for uninfected meadow and prairie vole males, suggesting that infection did not alter testosterone concentrations in these species (15–17). Although circulating estradiol concentrations among females were not related to parasite infection in the present study, female voles infected with *T. spiralis* had higher estradiol concentrations than male prairie voles, and female prairie voles had higher estradiol concentrations than female meadow voles.

Similar to studies of uninfected voles, circulating corticosterone concentrations were higher in prairie voles compared with meadow voles (15–17). Additionally, meadow vole females had higher corticosterone concentrations than conspecific males. These patterns of variation in corticosterone were not statistically related to differences in *T. spiralis* infection. Infection has been operationally defined as a stressor because infection can stimulate glucocorticoid release and can suppress immune function (31, 38). The corticosterone concentrations observed in this study were not higher than those previously reported for uninfected meadow and prairie voles, suggesting that infection did not serve as a chronic stressor for these animals (15–18). Additionally, the corticosterone concentrations reported for prairie voles in the present study are almost three times lower than stressor-induced values (33) and are not immunosuppressant (18). Although these data suggest that circulating corticosterone concentrations are not related to differences in *T. spiralis* parasite burden, they do leave open the possibility that corticosterone concentrations at the onset of infection may be related to differences in infection status 30 days postinoculation, because adrenalectomy before infection reduces subsequent numbers of *T. spiralis* larvae in mice (24).

Consistent with the finding that meadow voles have lower *T. spiralis* burdens than prairie voles, previous studies have demonstrated that meadow voles have higher humoral immune responses against keyhole limpet hemocyanin (KLH) than prairie voles (16, 17). The relationship between high humoral immunity and low parasite burden among meadow voles remains unspecified; however, surface antigens on *T. spiralis* larvae are cross-reactive with KLH (20). Additionally, antibody-mediated cytotoxicity is involved in the killing of newborn larvae during migration to muscle tissue (36). Taken together, these data suggest that stronger antibody responses to *T. spiralis* antigens in meadow voles may be one proximate mechanism mediating species differences in *T. spiralis* infection.

From an evolutionary perspective, the original Hamilton-Zuk (10) hypothesis predicted that parasite burden should be highest among individuals subjected to the most intense selection pressures (both intra- and intersexual selection). On the basis of this hypothesis, males should have higher parasite burdens than females, and individuals of polygynous species should harbor more parasites than individuals of monogamous species (10). Consistent with this hypothesis, sex differences were exaggerated among polygynous meadow voles, with males harboring more worms than females. In contrast to the predictions of the Hamilton-Zuk (10) hypothesis, polygynous meadow voles had lower *T. spiralis* burdens than monogamous prairie voles. However, these data support previous findings in voles and birds that illustrate higher parasite burdens among individuals of monogamous than polygynous species (14, 28).

The worm burdens reported in the present study are significantly lower than the worm counts previously reported for *Microtus* species; however, the overall trend is the same (i.e., prairie voles have higher worm counts than meadow voles; see Ref. 14). The difference
in worm counts between these studies may be due to variation in the percentage of viable larvae in an inoculum (Gamble, unpublished observations). In an effort to avoid this type of interexperiment variation, future studies should use larger numbers of animals to reduce variability. Additionally, differences in individual susceptibility to T. spiralis may also influence variation in worm counts and should be considered in future studies. Regardless of this variation, prairie voles are consistently more susceptible to T. spiralis infection than meadow voles (14).

Perspectives

Although these data suggest that the mating system may influence parasite infection, factors other than the mating system, such as diet, habitat, social behavior, and taxon, may also influence parasite infection. To assess the role of the mating system in sex and species differences in parasite infection, examination of parasite burden in species of other taxa is required. Future studies are needed to determine whether hormone and antibody concentrations alter or are altered by parasite infection by examining these proximate factors throughout the course of infection. In summary, these data suggest that both proximate and ultimate factors may influence sex differences in parasite infection.

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