Variation in levels of luteinizing hormone and reproductive photoresponsiveness in a population of white-footed mice (Peromyscus leucopus)


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Submitted 16 October 2009; accepted in final form 24 March 2010

Heideman PD, Pittman JT, Schubert KA, Dubois CM, Bowles J, Lowe SM, Price MR. Variation in levels of luteinizing hormone and reproductive photoresponsiveness in a population of white-footed mice (Peromyscus leucopus). Am J Physiol Regul Integr Comp Physiol 298: R1543–R1548, 2010. First published March 31, 2010; doi:10.1152/ajpregu.00686.2009.—Natural genetic variation in reproduction and life history strategies is a manifestation of variation in underlying regulatory neuronal and endocrine systems. A test of the hypothesis that genetic variation in luteinizing hormone (LH) level could be related to a life history trait, seasonal reproduction, was conducted on artificial selection lines from a wild-source population of white-footed mice (Peromyscus leucopus). Variation exists in the degree of suppression of reproduction by winter short-day photoperiods (SD) in wild-source individuals and in the laboratory population. In this population, most individuals from a photoperiod-responsive (R) artificial selection line are strongly suppressed reproductively in SD, while most individuals from a photoperiod-nonresponsive (NR) artificial selection line are only weakly reproductively suppressed in SD. We assayed levels of LH to test for genetic variation between lines that could contribute to variation in reproductive status in SD. Females from both lines were raised in long-day photoperiods (LD) or SD, ovariectomized under isoflurane anesthesia, and given estradiol implants. Levels of LH were significantly higher in the NR line than in the R line, indicating genetic variation for levels of LH. Levels of LH were higher in LD than in SD, indicating that levels of LH were sensitive to photoperiod treatment even with a controlled level of estradiol negative feedback. The results indicate that there is genetic variation in levels of LH that could be functionally important both in the laboratory in SD and in the wild population in winter. Thus genetic variation in levels of LH is a plausible causal factor determining winter reproductive phenotype in the wild population.

NATURAL POPULATIONS OF MAMMALS contain substantial interindividual variation in ecologically important reproductive traits, but the causes of that variation are mostly unknown (5, 8, 33). Genetic variation in reproductive traits provides the raw material for natural selection and evolution of reproduction. Reproduction and fertility are regulated by the hypothalamic-pituitary-gonadal axis (HPG axis) (11, 30, 31, 36). Thus genetic variation in the HPG axis could result in variation in reproductive phenotype in nature and provide raw material for the evolution of reproductive traits. Reproductive traits are regulated through environmental inputs affecting hypothalamic neurons that secrete gonadotropin-releasing hormone (GnRH), which in turn stimulates the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) from the anterior pituitary. LH and FSH are the major hormones directly regulating reproductive function, including gonadal development, gametogenesis, and levels of sex steroids. Genetic variation in levels of these hormones is a likely source of ecologically significant functional variation in reproductive phenotype. The nature and extent of such variation in natural populations are very poorly known, despite the importance of this variation in linking physiology with evolution and ecology (33, 39). A critical step is establishing a connection between natural variation in reproductive traits and genetic variation in hormone levels.

A commonly variable trait in rodents is suppression of reproduction in winter (33). Short winter photoperiods cause a long nightly elevation in pineal melatonin secretion that inhibits GnRH secretion, resulting in lower levels of LH and FSH that suppress gonadal function (2, 15, 34). Natural populations of temperate-zone rodents contain substantial functional and genetic interindividual variation in this trait (5, 8, 10, 17, 19, 33). Interindividual variation in the GnRH neuronal system has been correlated with variation in reproductive suppression (1, 18, 29), suggesting that variation in the GnRH neuronal system is related to interindividual variation in reproductive suppression in winter. Because GnRH neurons control LH secretion, interindividual variation in level of LH is a functional mechanism by which genetic variation in the HPG axis could directly affect reproductive phenotype.

In this study, we tested the hypotheses that 1) there is genetic variation in levels of LH in a wild-derived population of white-footed mice, Peromyscus leucopus, and 2) that this variation is related to genetic and phenotypic variation in reproductive photoresponsiveness. We predicted that levels of LH would be lower in a line of mice artificially selected for strong suppression of reproduction in short-day photoperiods (SD) than in a line of mice artificially selected against suppression of reproduction in SD. We tested levels of LH in both SD [8 h light (L8):16 h dark (D16)], a condition in which only one selection line was strongly reproductively suppressed, and long-day photoperiods [LD: 16 h light (L16):8 h dark (D8)], when both lines were fertile (19, 21). The hypothesis was tested in ovariectomized females with constant-release implants of estradiol from two divergent selection lines held in LD or SD.

MATERIALS AND METHODS

Development of selection lines. Mice were obtained from a wild-source laboratory colony at the Population and Endocrinology Labo-
enlarged ovaries, uteri, and presence or absence of visible corpora lutea (19). Females with ovaries ≤2 mm in length, lacking visible corpora lutea, and uterine diameter of ≤0.5 mm were classified as reproductively inhibited (R) by SD. Females with large ovaries (usually >3.5 mm in length), large visible follicles or corpora lutea, and uterine diameter >1 mm were classified as nonresponsive (NR) to SD. Males with a testis index (length × width of testis) < 24 mm² were classified as R; those with a testis index ≥ 32 mm² were classified as NR (17, 19). R males and females were paired in LD to produce offspring for the R line. NR males and females were paired to produce offspring for the NR line (19). Selection was continued on the offspring of each line to further develop the R and NR selection lines. The experiments in this study were conducted on mice from generations 7, 8, and 9.

**Pilot estradiol dose-response curve.** In a pilot experiment, intercapsular subcutaneous Silastic implants were tested for reproductive effects on female ovariectomized (OVX) mice in SD with different concentrations of estradiol (Fig. 1). Estradiol (E) implants were 1-cm lengths of Silastic 508-005 laboratory tubing (ID 1.02 mm, OD 2.16 mm; Dow Corning, Midland, MI) containing doses of 0.01, 0.04, 0.16, 0.64, 2.56, 6.40, or 10.24 μg of crystalline β-estradiol [1,3,5(10)-estratriene-3,17β-diol, CAS No. 1743-60-8; Sigma, St. Louis, MO] mixed in silicone rubber aqueous sealant (Perfecto Manufacturing; Noblesville, IN) (7). Females were anesthetized with inhalant isoflurane (Abbott Laboratories) and blood (100 μl) was collected in a heparinized capillary tube from a tail nick, with serum stored at −70°C until assay. Only samples collected within 2 min of the first touch of the mouse cage were used for analysis. At 3- to 5-day intervals, two additional blood samples were taken with the same protocol. These three samples (2 samples in 6 cases) were pooled for assay. Because LH release occurs in pulses that correspond to pulses of GnRH release, we considered that a pool from samples collected at intervals over a period of ~10 days would provide a better average for LH in an individual than a single point sample. Finally, 4 wk after the initial surgery (age 91 ± 7 days) mice were anesthetized with isoflurane and weighed, and a terminal blood sample was obtained by drawing blood from the orbital sinus until flow terminated. Blood samples were collected from individuals in random order with respect to treatment group and line during the period from 1000 to 1800. After the terminal blood collection, mice were euthanized with an overdose of isoflurane, and uteri were removed and weighed.

Assays for LH and E were conducted by the Ligand Core Lab of the University of Virginia. LH was measured with a two-site sandwich immunnoassay effective for multispecies use (26) with monoclonal antibodies against bovine LH [no. 581B7 (26)] and against the human LH β-subunit (no. 5303; Medix Kauniainen) as described in Ref. 16, including a tracer antibody (no. 518B7) kindly provided by Dr. Janet Roser (Department of Animal Science, University of California, Davis) and a capture antibody (no. 5303) biotinylated and immobilized on avidin-coated polystyrene beads (7 mm; Epitope Diagnostics, San Diego, CA). The assay standard was a mouse LH reference prep (AFP5306A; provided by Dr. A. F. Parlow and the National Hormone and Peptide program). The LH assay had a sensitivity of 0.04 ng/ml, an intra-assay coefficient of variation (CV) of 4.9%, and an interassay CV of 8.6%. In this experiment, LH was measured in a single LH assay. The E assay was a radioimmunoassay (DSL-4400, Diagnostic Systems Laboratories, Fullerton, CA; sensitivity was 10 pg/ml; intra-assay CV 6%, interassay CV 9.3%). In this experiment, E was measured in a single E assay. LH was analyzed in both the pooled sample and the terminal sample from each mouse. E was assayed in the terminal sample from most mice. For 13 individuals spread across all treatment groups, there was insufficient serum for E assay.
VARIATION IN LEVELS OF LH

Approval of animal subjects for this study was provided under protocol IACUC-104 from the Institutional Animal Care and Use Committee of the College of William and Mary.

Statistical analysis. Data were analyzed with JMP statistical software (JMP 4.0.4; SAS Institute, Cary, NC) on a Macintosh computer. The level of significance for all analyses was set at \( P < 0.05 \). General linear mixed models (GLMM) were used to test for significance of main effects (photoperiod, line, photoperiod \( \times \) line interaction, and implant) on levels of LH (pooled sample and terminal sample), ovary mass at the time of initial surgery, uterine mass at the end of the study, and levels of E in the terminal sample. To assess whether reproductive maturity (prepubertal or postpubertal) was a better predictor of levels of LH than line (NR or R), analyses were conducted in which reproductive maturity (as a random effect) replaced line as a main fixed effect. Finally, GLMM including photoperiod, selection line, and reproductive maturity were assessed for improved fit of the data to the model.

The data on levels of LH, ovary mass, and levels of E did not meet the assumption of equal variance among groups, and log-transformed data were used in the statistical analyses. In the initial analyses, body mass and age were included as covariates and generation number (7, 8, or 9) or family was included as a random effect in the analyses of LH, ovary mass, uterine mass, and levels of E. Only in cases in which the effects of these variables were significant were these variables included in the final analysis. These comprised one analysis in which age was significant as a covariate, one analysis in which body mass was significant as a covariate, and two analyses in which generation was significant as a random effect. In none of these did the addition or removal of the covariates or random effect factor alter the outcome for the main effects of line, photoperiod, interaction between line and photoperiod, or implant.

RESULTS

Body mass and ovary mass before implant. Body mass averaged between 18.1 and 18.9 g in all groups, with no significant effect of line, photoperiod, or other factors on body mass \( (P > 0.5 \) for all). Ovary mass at the time of ovariectomy (age 63 ± 7 days) was significantly related to line \( (F = 16.38; P < 0.0005) \) and photoperiod \( (F = 6.28; P < 0.05) \), with no significant interaction \( (F = 0.61; P > 0.4) \). Ovaries were larger in NR females than in R females in both photoperiods, with the lowest ovary mass in R females in SD (Fig. 2A). At that age, puberty had been completed in 100% of NR LD females and ~70% of NR SD and R LD females but fewer than 20% of R SD females (Fig. 2B), as indicated by the presence of corpora lutea or ovarian follicles that were visible macroscopically.

Confirmation of effects of E implants. Surgical E implantation increased circulating E, reduced levels of LH, and increased uterine mass relative to females with B implants. In females with E implants, there was no significant effect of photoperiod, line, interaction between photoperiod and line, or family on level of E \( (P > 0.50 \) for all). As expected, OVX females with blank implants had significantly lower levels of E than OVX females with E implants \( (OVX + B: 47 ± 11 \text{ pg/ml}; OVX + E: 147 ± 29 \text{ pg/ml}; F = 4.54, P < 0.05) \) and lower uterine mass \( (OVX + B: 9 ± 3 \text{ mg}; OVX + E: 27 ± 2 \text{ mg}; F = 20.98, P < 0.0001) \). NR females had significantly larger uterine mass than R females \( (NR: 31 ± 3 \text{ mg}; R: 22 ± 2 \text{ mg}; F = 4.40, P < 0.05) \), but there was no effect of photoperiod \( (F = 0.28, P > 0.5) \). Finally, OVX females with E implants had significantly lower levels of LH than OVX females with B implants. This was the case in both the pooled samples (level of LH in E implant: 0.79 ± 0.12 ng/ml, level of LH in B implant: 2.48 ± 0.45 ng/ml; \( F = 8.03, P < 0.01 \)) and the terminal LH sample (level of LH in E implant: 1.03 ± 0.19 ng/ml, level of LH in B implant: 2.39 ± 0.42 ng/ml; \( F = 9.74, P < 0.005 \)).

Effects of line and photoperiod on LH. In female mice raised in LD or SD and manipulated with OVX plus E, there were higher levels of LH in NR females than in R females and higher levels in LD than in SD in both the pooled and terminal samples (Fig. 3). In the pooled samples, there were significant differences in levels of LH by line \( (F = 17.83, P < 0.0001) \) and photoperiod \( (F = 32.69, P < 0.0001) \), with no significant interaction between line and photoperiod \( (F = 0.29, P > 0.5) \). Similarly, in the terminal samples groups were significantly different in levels of LH for line \( (F = 26.05, P < 0.0001) \) and photoperiod \( (F = 43.09, P < 0.0001) \), with no significant interaction between line and photoperiod \( (F = 0.08, P > 0.5) \). In the summary of the fit to the model, \( R^2 \) values were 0.48 and 0.52 for the pooled and terminal samples, respectively. Levels of LH measured from the same individuals with the two sampling protocols were highly correlated \( (R = 0.714, P < 0.0001) \).

Analyses of levels of LH using reproductive maturity (prepubertal or postpubertal) in place of selection line showed statistical outcomes similar to those using selection line. For both pooled and terminal samples, effects of both photoperiod and maturity were significant \( (P < 0.001 \) for all). GLMM using maturity in place of line provided similar fit to the model \( (R^2 \)
values of 0.50 and 0.49 for the pooled and terminal samples, respectively). The best fit model for levels of LH was provided by GLMM including line, photoperiod, and maturity ($R^2$ values of 0.58 and 0.62 for the pooled and terminal samples, respectively). In these models effects of photoperiod and line were significant ($P < 0.01$ for all), but the effect of maturity was significant only for the terminal samples ($P < 0.01$) and did not reach significance for the pooled samples ($P = 0.07$).

**DISCUSSION**

The differences in ovary mass and maturity in R and NR mice at the beginning of the study (Fig. 2) are consistent with previous results showing differences in reproductive photosensitivity of females between the selection lines (19, 24). E implants had significant effects on reproductive organ mass and levels of hormones. Females with E implants had an average uterine mass of 27 mg, typical of those at age 60 days in LD and in the appropriate size range for females in late stages of reproductive maturation (Fig. 1). Females with B implants had an average uterine mass of 9 mg, in the range of OVX females with the lowest doses of E in the pilot study (Fig. 1). Furthermore, females with E implants had significantly greater plasma E, significantly lower LH, and significantly greater uterine mass than females with blank implants. A potential concern in OVX females with E implants is the triggering of a daily rise in LH due to positive feedback near the time of the light/dark transition (9, 22, 35). However, levels of LH were higher in the OVX + B treatment group than in the OVX + E group, suggesting that the implants did not trigger positive feedback on LH at the times of sampling. The outcomes met our criteria to indicate that estrogen negative feedback on the reproductive axis was held constant at approximately the level that would be experienced by a female as it was entering the final stages of sexual maturation in LD.

In both photoperiods, levels of LH in the NR line were more than double the levels of LH in the R line (Fig. 3), indicating constitutively lower levels of LH in the R line relative to the NR line. Values for LH of individual mice from the pooled and terminal samples were highly correlated, suggesting that the two methods provided similar representations of levels of LH. Levels of LH were suppressed by SD in both lines, with LH in the R line in SD near zero (Fig. 3), indicating that SD affected the reproductive axis in both lines. However, as evaluated by ovary mass and completion of puberty (Fig. 2), only the R SD group was strongly reproductively suppressed. Photoperiod and pubertal status irrespective of selection line accounted for variation in levels of LH about equally as well as photoperiod and selection line. Despite the differences between the NR and R lines in both photoperiods, breeding records suggest that the R and NR lines do not differ in reproductive rate or litter size in LD (D. Broussard and P. D. Heideman, unpublished data). Levels of LH in the NR LD, NR SD, and R LD groups were all sufficient to permit puberty in most individuals, while those in the R SD group may be insufficient in most individuals (Fig. 3B). Overall, these results are consistent with the hypothesis that variation in reproductive photosensitivity between lines is due at least in part to constitutively lower levels of LH in the R line.

The gonadal sex steroids are part of a negative feedback pathway inhibiting LH and FSH release at least partially via inhibition of GnRH release (6, 12, 23, 28, 37). Previous work has suggested that enhanced sensitivity of GnRH neurons to the sex steroid negative feedback occurring specifically in response to the short photoperiods of winter may be one cause of winter suppression of fertility in seasonally reproducing mammals (14, 25, 32). Our results suggest that variation between the selection lines in reproductive photosensitivity is not due solely to differences in SD enhancement of sensitivity to steroid negative feedback, because, by definition, such differences should be induced only in SD and not in LD. Differences in levels of LH between the NR and R lines (Fig. 3) could be due to some combination of photoperiod-dependent changes in steroid negative feedback, constitutive differences in GnRH secretion, or differences in pituitary responsiveness to GnRH, as well as other unidentified causes. The fact that NR females with E implants had significantly larger uteri than R females with E implants suggests a difference in the sensitivity of reproductive tissue to estradiol stimulation. Similar variation in sensitivity to estradiol in other tissues could produce variation in other aspects of reproductive function.

Results from our population of white-footed mice (19) and from studies on a population of deer mice (Peromyscus maniculatus) by Blank and colleagues (3, 29) indicate substantial genetic variation in reproductive photosensitivity within natural populations. In white-footed mice, this study shows...
genetic variation in levels of LH (Fig. 3) that is correlated with genetic variation in reproductive photoresponsiveness. In the deer mouse population studied by Blank and colleagues, phenotypic variation in reproductive photoresponsiveness was also correlated with variation in levels of LH, with indirect evidence that the phenotypic variation has an underlying genetic basis (3, 29). Both sets of studies suggest that variation in levels of LH may be a cause of variation in reproductive function in wild populations. Furthermore, in both species there is variation in the GnRH neuron system that is a plausible cause for variation in levels of LH (1, 3, 29). However, many other factors also affect circulating levels of LH, including clearance rate, sensitivity of the pituitary to GnRH stimulation, number and secretary capacity of LH-secreting cells, and negative feedback directly on the pituitary (see Refs. 4, 20).

Insight into the cause of variation in levels of LH and reproductive function between lines could be obtained by testing pituitary responsiveness to challenge with exogenous GnRH, testing for direct correlations between LH levels and number of immunoreactive-GnRH neurons, or differences in levels of gonadal sex steroids in intact individuals.

Perspectives

Recent reviews have stressed the need for studies on individual and genetic variation in the endocrine system as a means to understand the functional significance of phenotypic variation (27, 39). This study provides such data on endocrine variation in relation to reproductive phenotype in the context of phenotypic variation that has functional significance in the wild population. This study does not provide information on the cause of divergence of the selection lines; replication of the selection lines would be required in order to distinguish the effects of artificial selection or random factors such as genetic drift (13). However, these results suggest that the wild population is likely to hold functionally important genetic variation in levels of LH. Our results suggest that genetic variation in levels of LH might account, at least in part, for variation observed in reproductive phenotypes in SD in the laboratory population and in winter reproduction in the natural population (19, 38).

Levels of LH would be valuable to measure in individuals taken directly from wild populations to assess functional relationships between endocrine variation and variation in reproductive phenotype.

ACKNOWLEDGMENTS

We thank Lydia Wright-Jackson for assistance with animal breeding and care.

GRANTS

This research was supported by a grant from the National Science Foundation (9875866) to P. D. Heideman, by a Howard Hughes Medical Institute grant through the Undergraduate Biological Sciences Education Program to the College of William and Mary, and by the College of William and Mary. In addition, we acknowledge National Institute of Child Health and Human Development (SCCPRR) Grant U54-HD-28934, University of Virginia Center for Research in Reproduction Ligand Assay and Analysis Core.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


