American Journal of Primatology 26:53–59 (1992)

BRIEF REPORTS

Urinary and Plasma Gonadotropin Concentrations in Golden Lion Tamarins *(Leontopithecus r. rosalia)*

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This paper describes the development and validation of a plasma and urinary gonadotropin immunoassay for golden lion tamarins *(Leontopithecus rosalia)*, an endangered New World callitrichid primate. The assay is derived from a macaque chorionic gonadotropin assay and was validated for both plasma and urine samples in *L. rosalia*. Levels of immunoreactive LH/CG in lion tamarin urine were highly correlated ($r = + 0.98$) with gonadotropin bioactivity. Immunoreactive LH/CG levels were examined in two contexts: in the urine of adult females and in the plasma of adult males after administration of estrogen. Peaks of gonadotropin excretion were detected in samples collected from nonpregnant adult females. The peaks occurred immediately prior to cyclic elevations in urinary estrogen excretion. Plasma LH/CG concentration in males measured 24 and 48 hours after a single 50 μg injection of estradiol benzoate were significantly lower than levels at these time points measured after control treatment. Together, the results of this study point to the utility of the gonadotropin assay for monitoring reproductive function in both female and male lion tamarins.

Key words: tamarin reproduction, urinary hormones, luteinizing hormone, negative feedback, ovulation detection

INTRODUCTION

The development of assays for urinary steroid hormone excretion in callitrichid primates, e.g., marmosets and tamarins [Hodges et al., 1979; Brand, 1981; Epple & Katz, 1983; French et al., 1983] have proven useful for evaluating ovarian function in females while minimizing the stress and disruption associated with blood collection. The dynamics of gonadotropin excretion in callitrichids is less well understood, and profiles exist for only a few species: *S. fuscicollis* [Hodges et al., 1979], *S. oedipus* [Ziegler et al., 1987], and *Cebuella pygmaea* [Ziegler et al., 1990]. Information about the temporal relationship between gonadotropin and estrogen excretion is essential for the detection and prediction of the periovulatory period in female callitrichids.

Received for publication June 22, 1990; revision accepted January 23, 1991.

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In this paper we report the use of a gonadotropin assay for plasma and urine samples from golden lion tamarins *(Leontopithecus rosalia)*. Two applications of the assay are described. In the first, we present profiles of urinary gonadotropin excretion in females and assess the relationship of gonadotropin peaks to the urinary estrogen cycle. In the second application, we evaluate the gonadotropin response to estrogen injection in male lion tamarins. In male common marmosets, an estrogen challenge yields significant elevations in plasma LH after an initial suppression [positive feedback, Hodges, 1980; Hodges & Hearn, 1978], while in other species only negative feedback (reduced gonadotropin concentrations) is noted after estrogen stimulation in intact males [Karsh et al., 1973; Westfahl et al., 1984]. We wished to evaluate which pattern is expressed by male lion tamarins.

METHODS

Urinary Gonadotropin Excretion and the Ovarian Cycle

Four females served as subjects in this experiment. Two were adult females (Fi and Mo; 12 and 13 years of age, respectively) who were housed in a social group with an adult male. The remaining females (n = 2) were daughters (Ge, 3 years old, and Ma, 10 months old) residing in family groups in the presence of their natal mother and father and related siblings. Sexual maturity occurs at 16–18 months of age in *L. rosalia* [French et al., 1989]. All tamarin family groups were housed in large cages (2.0 × 2.0 × 2.2 m) designed to encourage species-typical social and individual behavior. Female Fi was housed at the Brookfield Zoo, Chicago, IL, under similar conditions.

Urine samples were collected 3 to 7 times per week. One of two methods for noninvasive and nonstressful sample collection were used. In the first, large stainless steel pans were placed on the floor of the cage, and observers monitored the target female until she voided urine on the pans. In the second, a plastic shoebox-sized container was held under a target female until she provided a urine sample. Samples were divided into two aliquots, one for steroid assay and one for gonadotropin assay. Aliquots of urine for female Fi received several drops of 0.52 mol/l glycerol to preserve the protein hormones [Liversey et al., 1983]. All samples were stored at −35°C until assayed.

Gonadotropin Response to Estrogen Stimulation in Males

Four males, 3 to 4 years old, were selected for this experiment. Body weight ranged from 631 to 962 g (774.5 ± 69.6, mean ± s.e.m.). They were housed in pairs in separate cages, and maintained under conditions identical to the females described previously. The experimental protocol consisted of three phases. Males were injected with either 50 μg (s.c.) estradiol benzoate (EB) dissolved in corn oil (Phase II) or with oil alone (Phase I and III). Blood samples (0.5 to 2.0 ml) were collected from the femoral vein of restrained but unanesthetized animals immediately prior to the injection (0 hours) and 8, 24, 48, and 96 hours after injection. The 0 hour blood sample was collected at 0800 h in all phases. Serum was placed in heparinized tubes, kept on ice for 30 minutes, and spun for 30 min at 1500*g*. Plasma was stored at −40°C until assayed. Animals were given an iron supplement and several raisins after each blood draw. Samples at several time points were missed or were too small in volume for assay in Phase I, so quantitative comparisons of plasma LH concentrations were made for Phase II (EB) versus Phase III (Oil).

Hormone Assays

Estrogen. All urine samples were assayed for estrogen concentration with an RIA. The assay, which uses an estrone antiserum, trace, and reference standard,

measures both conjugated and unconjugated estrone, the predominant urinary estrogen in lion tamarin urine [French & Stribley, 1985]. Cross-reactivity of the antiserum was < 0.1% for estradiol and < 0.01% for estriol, progesterone, testosterone, and dihydrotestosterone. Samples of urine (5μl) were diluted to 5 ml (1:1000) with phosphate-buffered saline and then hydrolyzed by 25 μl β-glucuronidase (Type H-2, Sigma Chemical Co.), and extracted with diethyl ether. Hydrolysis efficiency has been previously estimated at 94% for estrone-sulfate and 100.3% for estradiol-glucuronide [French & Stribley, 1985]. Buffer blanks were less than 2 pg/tube. Intra-assay variability, based on duplicate sample agreement within each assay, ranged from 6.2% to 14.2%, and interassay variability based on a urine pool was 9.0% (n = 5). The estrogen concentration of each urine sample was divided by the creatinine concentration, which was estimated by the Jaffe method [Tietz, 1976].

Gonadotropin. Immunoreactive LH/CG in lion tamarin urine and plasma was measured using an assay system developed for macaque monkey chorionic gonadotropin (mCG) [Hodgen et al., 1974]. This assay has proven useful in evaluating CG in pregnant lion tamarins [Kleiman et al., 1978] and common marmosets [Hodgen et al., 1976]. The reagents included a primary antiserum to the beta subunit of ovine LH (H-26), iodinated human CG, and a House Reference Preparation of pregnant macaque urine (HRP 5/13/77) from the Wisconsin Regional Primate Research Center for the standard values. All urine samples were assayed at a volume of 100 μl during one assay run, and the intra-assay coefficient of variation, based on duplicates, was 9.6%. Interassay coefficient of variation was 15.5% (n = 10 assays). A single urine sample from a nonpregnant female was assayed at 4 volumes (12.5 to 150 μl), and a pooled sample of plasma from females in various reproductive conditions was assayed at two volumes (50 and 100 μl).

Sixteen urine samples were assayed for biological gonadotropin activity using a mouse dispersed interstitial cell testosterone (MICT) assay using the procedures outlined in Ellingwood and Resko [1980] and modified in Ziegler et al. [1987]. Samples were chosen from two females from the eight days prior to, eight days after, and the day of, peak concentrations in immunoreactive LH/CG activity.

RESULTS

Assay Validation

The mCG immunoreactive assay provided a good estimate of biologically active gonadotropin in female lion tamarin urine. Both assays detected peaks in gonadotropin excretion, and variations in nonpeak concentrations in one assay were paralleled by changes in the other. The correlation between gonadotropin concentrations measured by the bioassay and immunoassay was + 0.98 (n = 16 samples measured in both assays; $P < 0.001$). Serial dilutions of a female urine sample and a plasma pool from females produced binding inhibition curves that were statistically indistinguishable from the standard curve.

LH/CG Excretion Across the Estrogen Cycle

Three of the four females, all three years of age or older, exhibited cyclic patterns of urinary estrogen excretion. The fourth, Ma (10 months old) exhibited frequent, low concentration spikes of LH/CG and low, acyclic patterns of estrogen excretion. In all three of the cyclic females, prolonged elevations in estrogen excretion were generally preceded one or two days by spikes in LH/CG excretion (see Fig. 1). Although LH peaks were detected in all three females, the peaks in LH excretion were clearer in the female whose urine was collected on a daily basis and whose gonadotropin samples were frozen after the addition of glycerol to the sam-

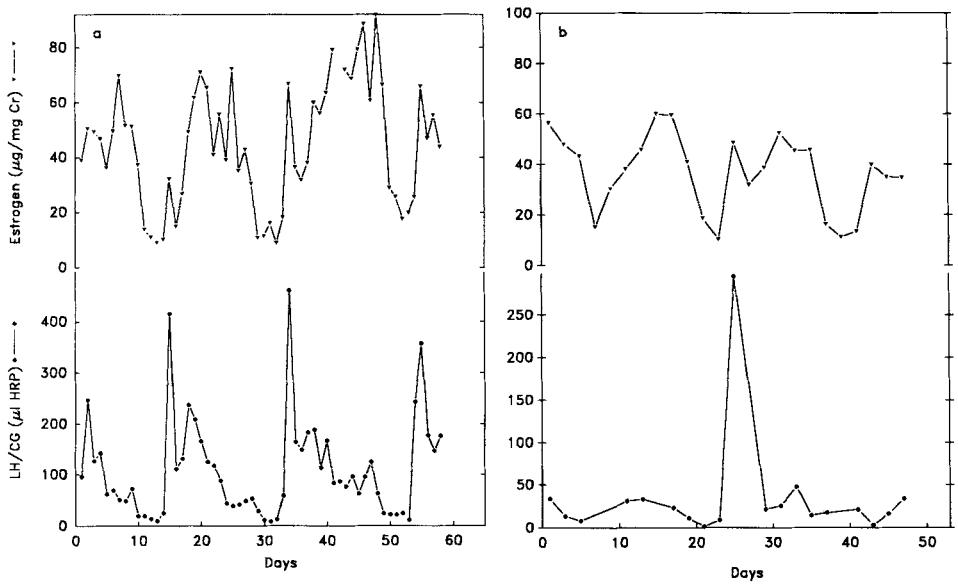

Fig. 1. Patterns of urinary LH/CG and estrogen excretion in two female lion tamarins: (a) Fi, 12-year-old adult female; (b) Ge, 3-year-old daughter in family group.

ple. In the 10-month-old female, estrogen concentrations were elevated on only one day, and changes in LH/CG excretion were not systematically related to changes in estrogen excretion.

Plasma Concentrations and LH Response to Estrogen Challenge

Injection of EB reduced plasma LH/CG concentrations 24 and 48 hours after injection in male lion tamarins. Figure 2 presents plasma gonadotropin concentrations after oil or EB injection. Mean plasma LH/CG concentrations remained relatively constant after oil injection, ranging from 9.35 ± 1.6 to 11.45 ± 1.4 μl HRP. LH/CG concentrations after EB injection ranged from 7.05 ± 1.4 to 11.7 ± 1.2 μl HRP. Analysis of variance of the LH/CG concentrations by hormone and time after injection revealed a significant interaction ($F(4,12) = 3.66, P = 0.036$). Post-hoc Tukey tests [Keppel, 1984] compared concentrations of LH/CG after EB injection with 0 hour concentrations. At no time point did mean LH/CG concentrations differ significantly from 0 hour in the Oil condition, but mean LH/CG values in EB-treated males were significantly lower ($P < 0.05$) than 0 hour concentrations at 24 and 48 hours. Mean LH/CG levels in EB-treated males were not statistically different from 0 hour values either at 8 or 96 hours after injection.

DISCUSSION

In this paper, we describe the use of a gonadotropin radioimmunoassay in lion tamarins. The assay, originally developed for macaque chorionic gonadotropin [Hodgen et al., 1974], correlates highly with a bioassay of gonadotropin activity. Periovulatory gonadotropin peaks in the urine of adult females are detectable with the radioimmunoassay, as is an estrogen-induced negative feedback LH response in the plasma of adult males.

The regular pattern of gonadotropin peaks *preceding* elevations in excreted

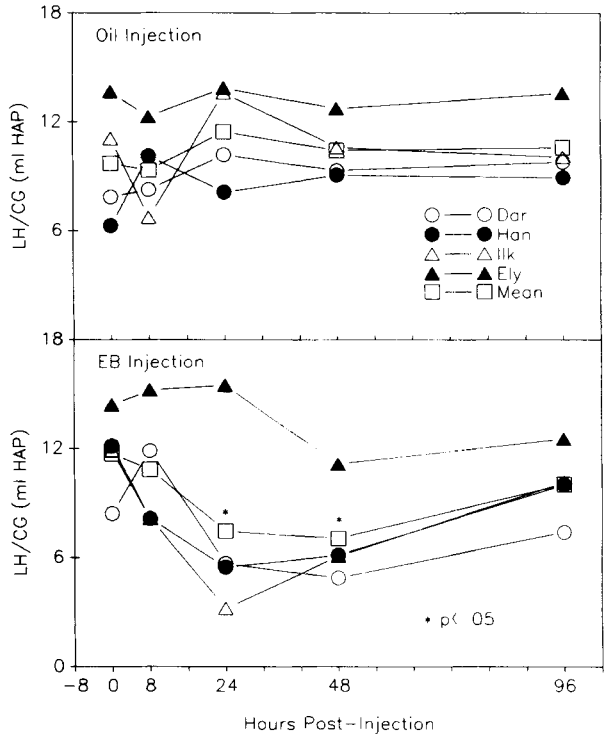

Fig. 2. Changes in plasma LH/CG concentrations in male lion tamarins in response to either oil (solid circle) or 50 μg injection of estradiol benzoate (EB, open circle). * = differs from 0 hour concentration, $P < 0.05$.

estrogen in female lion tamarins provides additional data on the atypical sequence of peptide/steroid hormone excretion across the ovarian cycle in marmosets and tamarins. In callitrichid primates, estrogen excretion profiles are characterized by sinusoidal patterns with long duration (6–10 day elevations in excreted levels) [*S. oedipus:* Brand, 1981; French et al., 1983; Ziegler et al., 1987; Heistermann, 1989; *Callithrix jacchus:* Hodges & Eastman, 1984; *S. fuscicollis:* Epple & Katz, 1983; *Leontopithecus rosalia:* French & Stribley, 1985; *S. bicolor:* Heistermann et al., 1987]. The relationship between the extended period of estrogen elevation and periovulatory endocrine dynamics has been a matter of considerable discussion [e.g., Hodges & Eastman, 1984; Ziegler et al., 1987]. In several other species, estrogen elevations occur immediately after spikes in urinary gonadotropin excretion [Eastman et al., 1984; Ziegler et al., 1987; 1990], suggesting that the estrogens reflect luteal function. The patterns of LH/CG and estrogen excretion in the lion tamarin presented in this paper are consistent with the interpretation that estrogen elevations are post-ovulatory events and that they occur after the LH peaks.

Urine samples were collected daily for one female and 3 times per week for the other females. Although LH/CG spikes associated with estrogen peaks were detectable for all females except the 10-month-old female, the peaks were more distinctive and occurred with each estrogen elevation for the female whose urine samples were collected daily. In addition, although peaks were detectable in the profiles of adult females, peak concentrations were higher in samples which contained the cryopreservative glycerol. This procedure has since become standard protocol for urine sample collection.

A negative feedback response of pituitary LH in response to a single 50 μg estradiol benzoate injection was demonstrated in male lion tamarins, with concentrations significantly lower than control values 24 and 48 hours after estrogen stimulation. No evidence of positive feedback was noted. This response is similar to that of intact male rhesus and cynomolgus macaques [e.g., Karsch et al., 1973; Westfahl et al., 1984]. In contrast to these findings, however, intact male common marmosets responded with increased plasma LH concentrations after a single injection of EB, with 2- to 3-fold increases 24 to 28 hours after estrogen administration [Hodges & Hearn, 1978; Hodges, 1980]. EB doses in our study were only slightly lower than those in Hodges' on a per kg basis, but it may be that larger doses are required to elicit positive feedback in intact male lion tamarins. Additional research on LH responses to higher and lower doses of EB in male lion tamarins and a demonstration of the effective dose for eliciting positive feedback in female lion tamarins are required to evaluate the nature of LH feedback responses in intact male lion tamarins.

CONCLUSIONS

1. Gonadotropin concentrations were measured in the urine and plasma of golden lion tamarins. An immunoassay designed to measure macaque chorionic gonadotropin (mCG) detected gonadotropin activity in the urine and plasma of females and in the plasma of males.
2. Measures of LH/CG bioactivity correlated highly ($r = + 0.98$) with immunoreactive LH/CG levels detected by the mCG immunoassay.
3. Peaks of LH/CG excretion were detected in 3 adult females. These probably reflect periovulatory gonadotropin surges, and they occurred immediately prior to prolonged rises in urinary estrogen excretion.
4. Males showed significant negative feedback gonadotropin responses 24 and 48 hours after administration of 50 μg estradiol benzoate.

ACKNOWLEDGMENTS

This research was supported by grant HD 23139 from the NIH to JAF, by grant RR 00167 from the National Institutes of Health to the Wisconsin Regional Primate Research Center (WRPRC), and by funds from the UNO University Committee on Research. The UNO Radioimmunoassay Laboratory was established in part with funds from the Nebraska Foundation. The antiserum H-26 was a gift from Dr. Gary D. Hodgen. We thank Ann Baker and Mark Warneke of the Chicago Zoological Society for assistance in sample collection and Betty Inglett for help in manuscript preparation. Toni Ziegler provided the impetus for us to conduct the project, facilitated our work at the WRPRC, and along with 4 anonymous reviewers, provided useful comments and criticisms of the manuscript. All experimental procedures described in this paper were approved by the UNO/UNMC Animal Review Committee (ARC #88-041-11). UNO has signed the Cooperative Research and Management Agreement for the Golden Lion Tamarin. JAF, WAD, and SEH are Regional Affiliate Scientists of the WRPRC. This paper is publication 29-017 from the WRPRC.

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