Testicular regulation of sexual dimorphisms in the ultradian profiles of circulating growth hormone in the chicken

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Ultradian patterns in plasma growth hormone (GH) concentrations were determined in adult white Leghorn roosters, hens and capons. Serial blood samples were collected every 15 min over 8 h through surgically placed chronic indwelling right atrial catheters and assayed for GH content by a homologous radioimmunoassay. In both sexes, GH levels fluctuated episodically, with peak and interpeak periods each lasting about 45 min in both roosters and hens. However, GH concentrations in the peaks and nadirs were 2.5–3.5 times greater in the plasma GH profiles of roosters as compared to hens, which resulted in roosters having higher mean GH concentrations. Caponizing completely feminized the episodic GH secretory profile. In contrast to chickens, the common sexually dimorphic feature in secretory GH profiles of mammals is the enhanced peak frequency found in females.

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Pituitary growth hormone (GH) has been shown to regulate several sex-dependent phenotypes in rats and mice. Growth rates (1-4), major urinary proteins (5-7) and several cytochrome P-450-dependent drug-metabolizing enzymes (8-11) demonstrate distinct gender-specific GH-dependent differences. Although the signal transduction mechanisms of GH action are poorly understood, the sexually dimorphic ultradian patterns of circulating GH have been shown to regulate these sex-dependent phenotypes (1-11).

Gender differences in systemic GH concentrations were reported first in the rat (12, 13), and has since been the most intensively studied species. In male rats, GH is secreted in episodic bursts every 3-4 h. Between the peaks, GH levels are undetectable. In female rats, the hormone pulses are more frequent and are of considerably lower magnitude than in males, whereas the interpeak concentrations of GH are always measurable (12-15). Apparently, exposure to the more continuous feminine secretory profile of GH produces the lower levels of major urinary proteins and hepatic drug metabolism, as well as reduced growth rates, found in female rats. Conversely, the episodic rhythm in GH secretion characterized as masculine is responsible for the occurrence of the several-fold higher levels of major urinary proteins, drug metabolism and enhanced growth rates seen in males (1-5, 7, 10, 11, 14, 15).

Sexual dimorphisms in GH secretory profiles also have been reported for mice (16, 17). In contrast to the rat, both the height and duration of the GH peaks and the average interpeak concentrations, which are barely detectable at best, are comparable in both sexes. Male mice, however, demonstrate a consistent periodicity of GH peaks approximately every 2.5 h, with interposed stable baseline concentrations that are significantly longer in duration than in females. The absence of extended interpulse baseline concentrations in females almost doubles the cycle frequency. Comparing endogenous GH patterns in mice and rats demonstrates that males of both species have less frequent pulses than females over the same time interval. This interpeak period, of what is essentially GH absence, appears to be a requisite signal for the expression of sex-specific GH-dependent traits in both species (3, 4, 9, 14, 16).

Components of the sexually dimorphic ultradian GH patterns defined in rats and mice appear to be found in humans. Despite overall circulating concentrations of much lower magnitude, plasma GH levels in men and women rise and fall, forming discrete peaks. In women, peak, interpeak and mean GH concentrations are higher and GH pulses occur more frequently compared with men (18, 19). In fact, GH peaks above a detectable threshold of 1 μg/l were reported to be three times more frequent in women (19).

Like mammals, some birds exhibit sexual dimorphisms in growth rates (20, 21) and hepatic drug metabolism (22), but the role of endogenous GH in regulating these gender-specific functions is unknown. Although the preponderance of GH studies in chickens has been limited to a single gender, a few investigators have reported sex differences in plasma GH concentrations of chickens (23) and turkeys (21, 24), but only lasting for a very short period in the young bird’s life. In fact, adult plasma concentrations of GH in chickens and turkeys...
may decline to 5–10%, or even lower, of prepubertal levels (24–26). In the present study, we have investigated the sexually dimorphic character of plasma GH levels in adult chickens when gender differences in hepatic drug metabolism are present (22). In addition, we have examined the effects of orchietomy to determine if testosterone-dependent regulation of avian growth rates and drug metabolism may be mediated by androgenic control of GH secretion, which is the case in mammals (9-11, 27).

Material and methods

Animals

Chickens were housed in the University of Pennsylvania Laboratory Animal Resources facility, under the supervision of certified Laboratory Animal Medicine veterinarians, and were treated according to a research protocol approved by the University’s Institutional Animal Care and Use Committee. Recently hatched white Leghorn chickens obtained from a commercial breeder were housed under a 12-h light: 12-h dark cycle and had free access to fresh feed and water. At 6 weeks of age, cockerels were castrated according to established procedures (28). At 13 months of age the animals were divided into experimental groups. Caponized birds exhibiting comb growth or radioimmunoassayable (29) plasma testosterone levels greater than those found in known capons were discarded.

Mobile catheterization apparatus

The mobile catheterization apparatus developed in this laboratory for repetitive blood sampling from unrestrained, unstressed and completely conscious rats (3) and mice (16, 30) was used in this study with the following modifications. The catheter consisted of a 17-cm long silastic (Dow Corning, Midland, MI) tube (0.64 mm inner diameter) attached at one end to a 150-cm long polyethylene (Clay Adams, Parsippany, NJ) tube (PE-20, 0.38 mm inner diameter). Two silastic segments 4-mm long (1.47 mm inner diameter) positioned on the silastic portion of the catheter, and two more segments of PE-190 (1.19 mm inner diameter) positioned on the polyethylene portion of the catheter, were used to anchor the catheter internally. Crimping in the catheter was prevented by using U-shaped (4-cm long) PE-190 cuffs at points where the direction of the catheter changed and a 10-cm long tube at points where the catheter was exteriorized. The entire length of catheter outside the animal’s body was protected by a metallic tether (SE-Extension Springs, 4.76 mm inner diameter; Hardware Products Company, Inc., Chelsea, MA). The mobile swivel unit was similar to that used routinely in our laboratory for rats (3), except the size of the mobile unit (12 cm) and the slit length on top of the metallic cage (1 m) were larger in our present study.

Surgery and plasma sample collection

Five animals from each group were implanted under pentobarbital-induced anesthesia (36 mg/kg body wt, iv) with chronic indwelling right atrial catheters. Details of the surgical placement of the catheterization apparatus have been described elsewhere (3, 30). Briefly, the silastic end of the catheter was introduced into the atrium through the jugular vein. After securing the cuffs to the vein and neck muscles, the catheter was exteriorized from the back and directed to the outside of the cage, protected within a metallic tether. The tether was anchored to the skin with a 2-cm sewing snap and stabilized by using a 5 × 7-cm plastic base (3, 30). Plasma corticosterone measurements documented the non-stressful advantage of this technique (30). Throughout the study, the chickens were maintained specifically pathogen-and medication-free.

Caponized chickens were allowed to recover for 4–5 days before being used for blood sampling. A 75-µl volume of blood was withdrawn every 15 min for 8 consecutive hours, centrifuged at 10 000 g and plasma collected in buffer (pH 7.6) containing 0.01 mol/l KPO4, 0.15 mol/l NaCl, 3.1 mmol/l Na2HPO4, 3.8 mmol/l EDTA, 0.2% (w/v) BSA and 0.4% (v/v) normal rabbit serum (Antibodies, Inc., Davis, CA) and stored at −80°C until assayed.

Growth hormone assay

Plasma GH concentrations were determined by our previously reported radioimmunoassay technique (14, 15) using materials supplied by Dr AP Parlow (Pituitary Hormones and Antisera Center, Torrance, CA). Iodinated cGH tracer (AFP7678B) was prepared with chloramine-T and then purified over a Sephadex G-100 (Pharmacia LKB, Piscataway, NJ) gel filtration column. Reference preparation chicken GH (AFP9020C) was used as “cold standard” for displacement curves. The primary (rabbit) antiserum (AFP551-11-1-86) was used at a final tube dilution of 1 : 400 000 in a total 500-µl incubate. After a 24-h incubation at 22–24°C, 200 µl of a 1 : 10 dilution of goat antirabbit immunoglobulin G (Antibodies, Inc., Davis, CA) containing 15% (w/v) polyethylene glycol 8000 was added and the bound form of GH was spun down at 2000 g for 30 min. To correct for non-specific binding, all GH concentrations of unknown samples were normalized to the background values obtained from equine plasma.

The intra-assay coefficients of variation (%) corresponding to plasma GH concentrations of 2.0, 4.0, 12.0, 16.0 and 32.0 µg/l were 1.4, 2.2, 1.8, 5.4 and 3.3, respectively. The interassay coefficients of variation (%) for the same GH concentrations calculated from 11 independent assay runs were 15.1, 14.6, 14.8, 12.7 and 12.1, respectively.
Data analysis and statistics

The ultradian patterns of changes in plasma GH concentrations obtained from serially collected blood samples were analyzed with the aid of the Cluster analysis computer program (31), as we have reported previously (16). Overall mean GH concentrations are not calculated by the Cluster program and were determined separately. Data obtained from the Cluster analysis program for male, female and capon chicken experimental groups were subjected to analysis of variance, and the differences were determined using t statistics and the Bonferroni procedure for multiple comparison.

Results

Cluster analysis of plasma GH concentrations obtained from serial blood collections of adult chickens revealed several gender differences in the episodic patterns of circulating GH (Table 1). Most prominent (Fig. 1) was the mean GH peak heights, which were more than three times greater in the rooster than the hens, and resulted in a similar sexual dimorphism in the peak areas. Although the peak amplitudes in the roosters occasionally exceeded 20 µg/l, they were found never to surpass 4 µg/l in the hens. The mean interpulse GH nadir of the rooster was 2.6 times greater than the average nadir found in the hens. In fact, the GH nadirs in the roosters were generally greater than the pulse amplitudes of the hens. [With an assay sensitivity of 0.6–0.8 µg/l, the low nadir values of the hens remained statistically (p < 0.01) measurable.] As a result of the greater nadir and peak heights of the rooster, the average plasma GH concentration was about 2.5 times greater in the rooster as compared to the hens.

The major similarity in the episodic GH secretory profiles in roosters and hens was the cycle frequency (Table 1). The lengths of the peaks and valleys were each about 45 min in both sexes, resulting in a similar cycle frequency of approximately 1.5 h for roosters and hens. Whereas the plasma GH nadirs and peak heights were sexually dimorphic, the percentage increase from nadir to peak was fairly similar in roosters (108%) and hens (70%) and represented a statistically significant (p < 0.01) increase in both sexes.

Caponizing resulted in a complete feminization of the episodic GH secretory profile (Table 1). The similarities in the capons' and hens' average plasma GH concentrations, peak heights and areas, as well as nadirs, resulted in their circulating GH profiles being indistinguishable but quite different from that of the rooster (Fig. 1).

Discussion

The present study demonstrates a sexual dimorphism in the plasma GH profiles of adult chickens. Whereas GH levels were higher in the peaks and interpulse nadirs, resulting in a greater average GH concentration in roosters as compared to hens, there were no gender differences in the frequencies of the peaks and nadirs. In comparison to previous findings, GH levels within the ultradian profiles of our adult layer hens were the same as those reported for 12-week-old broiler pullets (26). Another study analyzing plasma GH profiles in adult roosters (25) reported pulse and interpulse baseline GH concentrations to be about twice as great as reported here. In agreement, however, was the percentage increase from nadir to peak and the pulse frequency of the roosters' GH profile (25). Lastly, in an earlier study comparing males and females it was reported that broiler chickens exhibit a sexual dimorphism in circulating GH profiles only during weeks 6–8 of life, at which time GH secretion in the cockerel was pulsatile, while pullets had no measurable systemic GH levels (23). Unfortunately, this later study was concluded when the birds were 60 days of age.

We have found that orchiectomy early in life* produces a complete feminization of the circulating GH

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*Owing to the highly diffuse morphology of the chicken gonads, ovariectomies are virtually impossible at any age and orchiectomies tend to be limited to the first 2–8 weeks post-hatching, when testicular boundaries are more definitive (26). Unfortunately, effects of orchiectomy early in life are not always reversible by androgen replacement (22, 33) and thus may not reflect similar changes produced by adult orchiectomy, were the procedure possible.
Fig. 1. Individual and representative plasma growth hormone profiles in adult white Leghorn roosters, hens and capons (two of each). Plasma concentrations of growth hormone were determined by radioimmunoassay in serial samples collected from surgically placed chronic indwelling right atrial catheters.
patterns of the capon. In contrast, it has been reported that plasma GH levels are unresponsive to either castration at 40 days of age or to testosterone administration through 147 days of age (34). However, in this cited study the determination of GH at a single time point, once every two weeks, could have failed to detect more subtle effects apparent from our serial blood collections. In support of our findings are reports that early orchietomy permanently feminized plasma GH profiles in rats (27).

In agreement with mammalian studies (12−15), we have found that GH secretory profiles are episodic and sexually dimorphic in adult chickens. Although there is a great variation in the amplitudes and frequencies of the GH peaks and interpeak periods between mammalian species, a common element defining the sexual dimorphism is the greater pulse frequency in the female. In fact, we have reported that this gender difference in pulse frequency is the “signal” in the plasma GH profile of rats and mice that regulates sex-specific differences in both growth rates and cytochrome P450-dependent drug metabolism (3, 4, 16). In contrast, the pulse frequency is the one element in the circulating GH profiles that is the same for roosters and hens. Although it is possible that GH-dependent, sex-specific traits in the chicken are regulated by signaling elements other than pulse frequency (i.e. pulse or nadir amplitudes), it should be noted that a GH profile of infrequent daily pulses characteristic of male rats, mice and humans was highly effective in increasing longitudinal bone growth and mass as well as reducing carcass fat in pullets, whereas the continuous-like pattern of GH secretion found in female mammals was ineffective (35).

The finding of statistically significant gender differences in the circulating GH profiles of the adult chicken does not by itself demonstrate a biological role for this dimorphism. With the exception of humans (19), serially measured plasma GH levels in other mammals (14, 16) are considerably higher than in adult chickens. And while circulating GH concentrations may be comparable in humans and adult chickens, the $K_d$ (i.e. affinity) of the GH receptor for GH is about $1 \times 10^{-9}$ mol/l for humans (36–38) and approximately $4 \times 10^{-9}$ mol/l for chickens (39, 40), indicating that chickens require a fourfold greater plasma concentration of the hormone than humans to achieve a similar half-maximal saturation of the receptor. Nevertheless, adult chickens do exhibit a sexual dimorphism in drug metabolism (22), and comparable gender differences in mammals have been shown to be regulated by sex-specific profiles in circulating GH (4, 9, 11). Unfortunately, very little is known about GH regulation of avian functions, and even less is known about the importance or even the occurrence of sex-dependent differences in avian GH secretion. Until more information is learned about this subject, any conclusions regarding the role of gender-specific avian GH profiles in regulating sexually dimorphic functions remain speculative.

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