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Vasotocin receptor expression in the brain and pituitary gland during the ovulatory cycle of the fowl

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ABSTRACT

Vasotocin receptors are members of seven transmembrane spanning G-protein associated receptors. Several isoforms have been recognized in mammals and birds. It has been shown that VT-1 expression occurs primarily in the brain while VT-2 expression occurs mainly in the pituitary. There is no current evidence to support that both VTR-1 and -2 are found in a single tissue. Our goal in this experiment was to see if VT-1 and VT-2 receptor mRNA expression varied in known sites of expression over the period of the ovulatory cycle of broiler breeder hens. In order to study potential changes in VT-1 and VT-2 expression, birds were sacrificed at 3 hour intervals over a 24 hour period. Blood samples were drawn. After cervical dislocation, the brain, pituitary, shell gland, and kidney were removed. Plasma was stored at -20ºC prior to determination of corticosterone levels by radioimmuno assays. Isolated mRNA from the brains and the pituitaries was transferred to nylon membranes for northern slot blot analysis. cDNA for VT-1 and VT-2 was used to make random primed cDNA probes. Corticosterone levels significantly increased at 9 hours post oviposition relative to all other times. Neither VT-1 or VT-2 expression showed any significant variation over the 24 hour cycle. Based on these results, we conclude that VT-1 and VT-2 steady state mRNA levels do not fluctuate dramatically over the ovulatory cycle of broiler breeder hens. Further work on membrane bound receptors and on circadian variations in membrane bound receptors in the brain and pituitary is currently underway of broiler breeder hens.

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INTRODUCTION

The effects of the circadian (~24 hr) rhythms on endocrine, reproductive, and immune system function in mammals and birds have been studied for many years. One circadian rhythm that is unique to certain birds, and laying hens in particular, is the daily production of a hard shelled egg. While the study of relationships among the various hormones, their receptors and the central regulation of hormone secretion has progressed dramatically over the past 10 to20 years, this research has lagged behind in avian species relative to mammals. Recently, we have been working with Larry Cornett, Department of Physiology, University of Arkansas Medical School and Dennis Baeyens, Department of Biology, University of Arkansas, Little Rock on the cloning and characterization of two new "novel" hormone receptors in the chicken. These receptors bind the small peptide hormone arginine vasotocin (AVT), an nonapeptide (nine amino acids), that is the avian homolog to mammalian arginine vasopressin (Hadley, 1996; Tan et al., 2000).

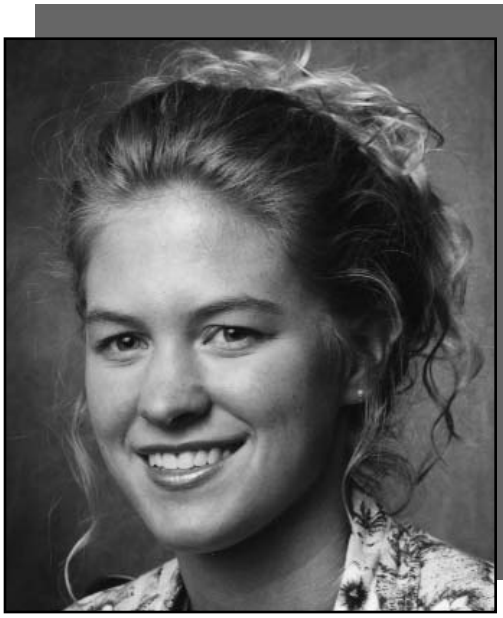
Arginine vasotocin has been shown to have anti-diuretic effects on the kidney (increased water retention, decreased urine production, and increased blood volume) and is the primary anti-diuretic hormone in chickens (Scanes, 2000). Further, AVT has additional functions that are similar to those associated with a second hormone, oxytocin, in mammals. These functions include the profound stimulation of uterine smooth-muscle contraction associated with oviposition (egg laying) and ejaculation (Rzasa, 1984). Finally, AVT has also been implicated in the stimulation of pituitary prolactin secretion, which regulates broody (mothering) behavior (El Halawani et al., 1992) and of adrenocorticotrophic hormone (ACTH), which stimulates the adrenal glands to produce corticosterone i.e., the primary glucocorticoid hormone associated with stress in birds (Castro et al., 1986).

The site of AVT synthesis is the neurohypophysis, the neuropeptide secreting portion of the posterior pituitary gland located just below the hypothalamus on the bottom surface of the brain. This hormone is then secreted into the circulation via a diffuse capillary bed where it travels throughout the body or, alternatively, AVT can be secreted into the intracellular spaces associated with the adenohypophysis (anterior pituitary gland) to rapidly affect the production of pituitary hormones such as prolactin, ACTH, luteinizing hormone (LH), and follicle stimulating hormone (FSH). The receptors for AVT, VT-1 (Tan et al., 2000), and VT-2 (Baeyens, Cornett, Vizcarra, and Kirby, unpublished)

Meet the Student-Author

I was born in Fayetteville and graduated from Rogers High School, which is where my family currently lives. I am a sophomore at the University of Arkansas. I am a member of Gamma Beta Phi and work with the Baptist Student Union, including being a part of their housing ministry. I have been awarded a University Scholarship, a Poultry Science Scholarship, and a Tyson Memorial Scholarship. My freshman year, I was on the Dean's List and in Spring 2001 made the Chancellor's list with a 4.0 grade point average.

I hope to pursue a career in medicine, either as a nurse or a doctor. My love for medicine stems from a desire to help people. My goal is to become a missionary overseas where there is a desperate need for more people in the medical field. I feel that helping people with their health gives me a chance to share with them the love of God. It is through medicine that I feel I will best be able to use the skills that God has given me and express the love for people that I have.



Kelly R. Shaffer

have been cloned, sequenced, and expressed in vitro. In preliminary work it has been shown that the VT-1 receptor is expressed in the brain and oviduct and that the VT-2 receptor is expressed in the pituitary of hens (Baeyens, Cornett, Vizcarra and Kirby, unpublished). However, the temporal, spatial, and functional differences between these receptors have not been determined.

Our objective in this research was to characterize the circadian, or ovulatory cycle, variation in tissue specific mRNA expression for VT1 and 2 in the context of fluctuations in plasma corticosterone, LH and FSH.

MATERIALS AND METHODS

Sexually mature broiler breeder hens (Cobb 500) were placed in individual cages on the U of A Poultry Farm and maintained on a daily schedule of 16 hours of light and 8 hours of dark with lights on at 0600 hours and lights off at 2200 hours. The time of oviposition was recorded for each hen in the experiment, and all times are expressed as hours relative to the recorded oviposition (e.g., +3 hours, 3 hours after an egg was laid).

Experimental. At three-hour intervals, beginning at time 0 (oviposition) and ending 21 hours later, four hens were selected per time point and bled by venipuncture with plasma collected and frozen for subsequent radioimmunoassay (RIA) determination of corticosterone, FSH, and LH. After blood sampling, hens were killed by cervical dislocation and the brain (principally hypothalamus), pituitary, and kidney were removed and frozen in liquid nitrogen for total RNA isolations.

RNA Analysis. Following the completion of all tissue collections, total RNA was isolated, separated by electrophoresis and transferred, or in the case of pituitaries, slot-blotted, to a nylon membrane using standard procedures (Sambrook, et al., 1989). Filters were then sequentially probed with P32- labeled, randomly primed cDNA probes for VT-1, VT-2, and a 28S ribosomal RNA probe. Relative expression of each RNA was then quantified using a Typhoon phosphor imaging system. Relative levels for VT-1 and VT-2 expression were expressed as a percentage of the 28S ribosomal RNA control. Relative RNA expression levels were analyzed using the General Linear Models procedure in SAS.

Radioimmunoassays. Luteinizing Hormone (LH) concentrations were measured by RIA using reagents provided by the US Department of Agriculture-Agricultural Research Service Animal Hormone Program. Concentrations of Follicle Stimulating Hormone (FSH) (Krishnan et al., 1993) in plasma (150 _l) and pituitary gland extracts were quantified in duplicate by RIA using reagents provided by the USDA/ARS. Concentrations of corticosterone in the plasma were quantified by RIA similar to that previously described (Proudman and Opel, 1989) with reagents provided by Dr. John Proudman, USDA-ARS/GGPL, Beltsville, Md. Changes in plasma and pituitary hormone concentrations were analyzed using the General Linear Models procedure in SAS 2000.

RESULTS AND DISCUSSION

Northern analysis revealed expression of mRNA for VT-1 in the brain, for VT-2 in the pituitary, and expression of neither VT-1 nor -2 in the kidney. Upon inspection of these steady state mRNA levels, no circadian or ovulatory cycle state variations in either VT-1 nor VT-2 receptor steady state levels were detected. These results suggest that if variations in AVT receptor levels are associated with circadian or ovulatory cycle changes, these changes are independent of receptor mRNA level. Alternatively, as steady state mRNA levels can not identify changes in coupled transcription rate, translation efficiency, or mRNA stability, a different assay may have provided better insight. Furthermore, until an effective receptor binding assay is developed for the VT-2 receptor, it will be difficult to assess receptor protein levels or function.

While the VT-1 and VT-2 mRNA levels failed to show any significant variation in expression over the 24-hour sampling period, plasma levels of corticosterone showed a clear circadian pattern of variation (Fig. 1). The pattern shown in Fig. 1 is the observed peak in plasma corticosterone concentrations approximately 9 hours after oviposition (and about 12 hours after lights on). This is quite different from the pattern observed in humans, with the highest observed levels of glucocorticoids occurring within 1 hour of exposure to light (reviewed in Griffen and Ojeda, 1988). This peak in plasma corticosterone occurred in the absence of any observed change in AVT receptor expression; it coincided with the nadir in plasma FSH and precedes that of plasma LH by one sampling period. As the inhibitory effects of elevated corticosterone levels are

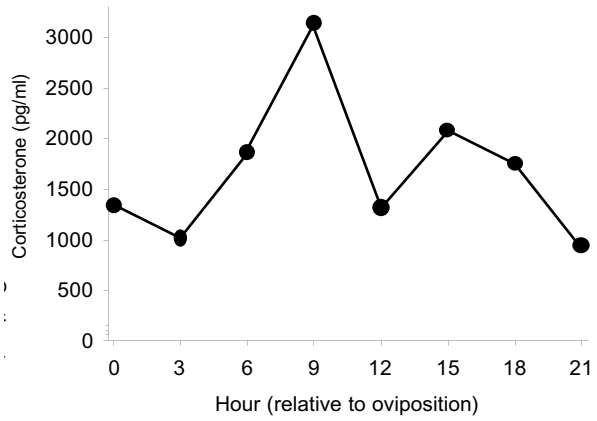


Fig. 1. Plasma concentrations of corticosterone in laying broiler breeder hens over an ovulatory cycle. The circadian rhythm of corticosterone is evidenced by the significant ($p<.05$) elevation in plasma concentration observed at 9 hours post oviposition. Hens (4 per time point) were selected and killed at three-hour intervals relative to oviposition; all hens represented would have produced an egg on the day following sampling as well. Blood samples were collected within 2 minutes of capture, and corticosterone levels were determined by radioimmunoassay.

well documented, this observation fits the previously described experimental results (Griffen and Ojeda, 1988; Hadley, 1996).

As documented previously by (Krishnan et al., 1993), plasma FSH levels vary by about two-fold over the ovulatory cycle (Fig. 2), with the peak levels of

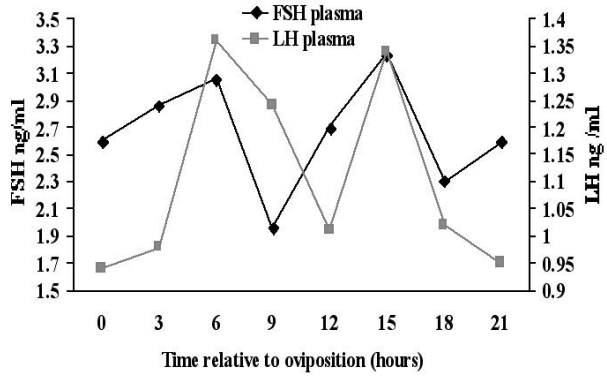


Fig. 2. Plasma concentrations of FSH (diamonds) and LH (squares) in laying broiler breeder hens over an ovulatory cycle. The ovulatory rhythms of FSH and LH are evidenced by the significant ($p<.05$) elevation, decline and elevation again in plasma concentrations observed between six and fifteen hours post-oviposition. Hens (4 per time point) were selected and killed at three-hour intervals relative to oviposition; all hens represented would have produced an egg on the day following sampling as well. Blood samples were collected within 2 minutes of capture, with LH and FSH levels determined by homologous radioimmunoassay.

plasma FSH occurring during the period of follicular recruitment (<9 hrs post oviposition) and immediately before the peak of the preovulatory LH surge at 16-20 hours post oviposition. Observed LH levels were somewhat lower in this experiment than has been described for the ovulatory cycle in leghorn chickens (Johnson and van Tienhoven, 1980); whether this is due to the use of a different assay, standards, or fundamental differences between leghorns and broiler breeders cannot be determined from this data. The sampling interval used here, 3 hours, more than likely missed the peak of the preovulatory LH surge (Fig. 2), however, the observed significant increase in LH suggests that the hens would have ovulated an egg on the morning of sacrifice. These daily variations in plasma LH and FSH concentrations have been shown to be critical in the regulation of the ovulatory cycle of the hen (Johnson, 2000).

Pituitary contents of FSH and LH (ng/mg tissue) showed considerable variation over the ovulatory cycle as well (Fig. 3). These variations in pituitary contents

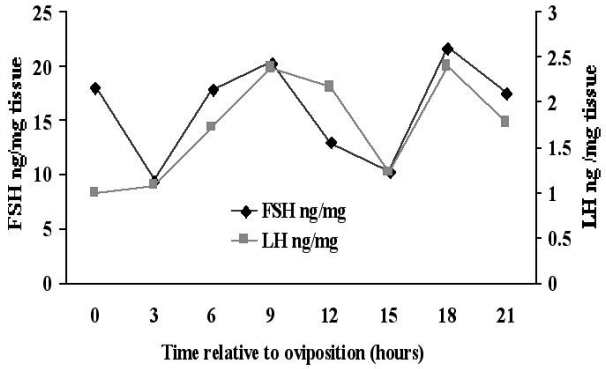


Fig. 3. Pituitary contents of FSH (diamonds) and LH (squares) in laying broiler breeder hens over an ovulatory cycle. The ovulatory rhythms of FSH and LH are evidenced by the significant ($p<.05$) decline, elevation and decline again in pituitary contents observed between three and fifteen hours post oviposition. Hens (4 per time point) were selected and killed at three-hour intervals relative to oviposition; all hens represented would have produced an egg on the day following sampling as well. Pituitaries were removed immediately following death, frozen and homogenized, with LH and FSH levels subsequently determined by homologous radioimmunoassay.

are most likely associated with either increased synthesis of hormone, decreased rate of secretion or turnover, or some combination thereof. We have observed that variations in both plasma and pituitary concentrations of both hormones follow a similar pattern, when we

advanced pituitary contents by 3 hours, we saw that the data coincide. The changes observed in pituitary hormone contents were preceded by changes in plasma hormone levels by about 3 hours (data not shown). These results suggest that rates of secretion, as suggested by plasma hormone concentrations, account for much of the variation observed in pituitary contents. That is, increased secretion leads to higher plasma concentration and reduced pituitary content. As these samples were collected from different individuals at each time point, these data provide insight into the robustness of the daily ovulatory cycle.

In summary, we were unable to demonstrate any circadian variation in the pattern of AVT-receptor mRNA expression in either the brain or pituitary at the gross level. Whether or not finer variations in mRNA expression occur or whether variations in functional receptor levels vary over the ovulatory cycle cannot be determined from this study. However, the hens studied clearly demonstrated daily rhythms of corticosterone, FSH, and LH. An interesting relationship between pituitary LH and FSH contents and plasma levels of these hormones was observed, with pituitary levels changing approximately 3 hours after observed changes in plasma levels. Further work to characterize changes in AVT-receptor expression changes will be needed to more completely answer this question.

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