Physiology and Endocrinology: Neuroendocrinology and Hormone Receptors

704 Chicken Pit-1 isoforms: Expression, nuclear localization, and involvement in growth hormone promoter activation. M. Mukherjee* and T. E. Porter, *University of Maryland*, *College Park*.

A POU-Homeodomain transcription factor, Pit-1, is expressed in lactotrophs, thyrotrophs and somatotrophs of the anterior pituitary gland. Pit-1 regulates expression of Prolactin, thyroid-stimulating hormone and growth hormone (GH). Multiple isoforms of Pit-1, differing from each other primarily in the N-terminal transactivation domain, have been reported. In chickens, 4 Pit-1 mRNA isoforms have been reported, Pit-1α, Pit-1β1, Pit-1β2 and Pit-1γ, but functional assays of these isoforms have not been completed. This study aimed at characterizing each isoform in its ability to translocate to the nucleus and regulate the chicken GH gene. We hypothesized that the isoforms will differ in their ability to translocate to the nucleus and/or regulate the GH gene, due to the presence of different transactivation domains. Expression of all isoforms from recombinant expression plasmids in HEK-293 cells was confirmed by Western blotting using antiserum against rat Pit-1. Pit-1α and Pit-1β2 were found to activate the GH promoter, while Pit-1γ had no effect. Interestingly, the level of expression of Pit-1γ was considerably lower than the other isoforms. Nuclear localization of the isoforms was tested in HEK-293 cells using immunofluorescence localization of expressed proteins. All isoforms except Pit-1γ showed efficient nuclear localization. To eliminate the possibility that lower expression of Pit-1γ was due to translational inefficiency, N-terminal HA- and c-myc-tagged proteins were expressed in HEK-293 cells. All tagged isoforms, except Pit-1γ were detected using antibodies directed against the tag, indicating that reduced expression of Pit-1γ was not due to inefficient translation. A remaining possible explanation for the reduced Pit-1γ levels observed is proteolytic degradation of an unstable Pit-1γ, a hypothesis that we will test empirically using inhibitors of protein degradation pathways. Future work will focus on elucidating potential physical interactions between the isoforms and their ability to interact with other transcriptional co-activators involved in regulation of Pit-1 regulated genes.

Key Words: transcription factor, anterior pituitary, growth hormone

705 Ras-dva is a novel Pit-1 and glucocorticoid regulated gene in the developing avian pituitary gland. L. E. Ellestad* and T. E. Porter, *Department of Animal and Avian Sciences and Molecular and Cell Biology Program, University of Maryland*, *College Park*.

Corticosterone (CORT) initiates growth hormone (GH) and prolactin (PRL) expression during embryogenesis. Microarray screens identified Ras-dva as a glucocorticoid-induced gene that may play a role in regulating GH and PRL expression. The objective of this study was to characterize tissue-specific and glucocorticoid regulation of Ras-dva expression in the developing chick embryo. Pituitary Ras-dva mRNA increased from embryonic day (e) 10 to a maximum just before hatch, then decreased post-hatch ($P < 0.05$; n = 3). Ras-dva mRNA was highly enriched in the pituitary relative to other tissues ($P < 0.05$; n = 3). CORT increased Ras-dva mRNA in mid- and late-stage embryonic pituitary cells, both in the presence and absence of a protein synthesis inhibitor ($P < 0.05$; n = 3), suggesting it may be a direct target of the glucocorticoid receptor (GR). We identified 5 putative Pit-1 binding sites (−0.35, −2.2, −2.5, −3.2, and −3.4kb) and 2 putative GR binding sites (−2.1 and −4kb) within the 5′-flanking region of the chicken Ras-dva gene that may be responsible for pituitary specificity and glucocorticoid regulation. E11 pituitary cells were transfected with reporter constructs containing 2kb (pGL3–2kb) and 4kb (pGL3–4kb) of the 5′-flanking region and cultured in the presence or absence of CORT (n = 3). Under basal conditions, pGL3–2kb was activated 40-fold over an empty reporter vector ($P < 0.05$), indicating that the most proximal Pit-1 site may be responsible for basal promoter activity in embryonic pituitary cells. Mutagenesis of this site in pGL3–2kb substantially reduced basal promoter activity ($P < 0.05$), confirming that this Pit-1 site is necessary for full activation of the promoter. CORT treatment had no effect on pGL3–2kb activity, but increased activity of pGL3–4kb 5-fold ($P < 0.05$). Surprisingly, mutagenesis of the potential GR binding sites did not affect CORT induction, indicating that neither putative GR binding site is necessary for CORT stimulation. In conclusion, Ras-dva is a novel glucocorticoid-regulated gene in the developing pituitary that is expressed in cells of the Pit-1 lineage, including GH- and PRL-producing cells.

Key Words: growth hormone, prolactin, corticosterone

706 Hypothalamic galanin-like peptide and kisspeptin may regulate the hypothalamo-pituitary-gonadal axis in the Mallard duck (*Anas platyrhyncos*). G. S. Fraley*, *Hope College*, *Holland, MI*.

The Mallard duck is a seasonal breeder and an excellent model for studying the neural mechanisms that regulate the activation of the hypothalamo-pituitary-gonadal axis (HPG). Recently, 2 neuropeptides have stood out as important modulators of the mammalian HPG, namely kisspeptin (KP) and galanin-like peptide (GALP). The goals of these studies were to determine (a) if KP and GALP regulate the avian HPG, (b) if KP and GALP are expressed in the mallard brain, and (c) if KP and/or GALP are co-localized with aromatase in the avian brain. Central administration of both KP and GALP significantly (ANOVA; $P < 0.01$) increased plasma luteinizing hormone, an effect blocked by pretreatment with the GnRH antagonist, acyline. Kisspeptin and aromatase immunoreactive (ir) cell bodies were observed in the medial preoptic nucleus (POM) and in fibers throughout the anseriforme brain. Virtually all POM kisspeptin-ir soma also expressed aromatase, suggesting that autocrine mechanisms may predominate in the interaction between steroid provision and kisspeptin expression. No colocalization was observed between kisspeptin-ir and GnRH-ir, although the respective fibers were in dense close proximity throughout the tuberoinfundibular area. GALP cell bodies were observed in the tuberoinfundibular nucleus and GALP-ir fibers were observed in close proximity to both GnRH- and KP-ir cell bodies and fibers. Taken together, these data suggest that estradiol synthesized by aromatase- and kisspeptin co-expressing POM neurons may regulate the HPG via an effect on GnRH secretion. Furthermore, as is observed in mammals GALP is anatomically positioned to regulate the HPG via interactions with both GnRH and KP. These observations suggest a conservation of HPG regulation in birds and mammals.

Key Words: luteinizing hormone, KiSS1

707 Gene expression profiling of dopamine-melatonin neurons in the avian premammillary nucleus. S. Kosonsiriluk*, S. W. Kang, L. J. Mauro, J. R. Garbe, S. C. Fahrenkrug, and M. E. El Halawani, *University of Minnesota*, *St. Paul*.

Dopamine-melatonin (DA-MEL) neurons of the hypothalamic premammillary nucleus (PMM) are proposed as a site for photoperiodic time measurement regulating reproductive seasonality in birds. Gene expression profiles of PMM DA-MEL neurons from photosensitive long day

(LD; n = 4 pools with 6 birds/pool) and short day (SD; n = 4 pools with 6 birds/pool) turkey hens were determined at circadian time 14 (CT14). The chicken 20.7k long oligo microarrays purchased from the University of Arizona were validated and used for this study. After hybridization and image acquisition, fluorescence intensities were extracted and graded using BlueFuse software (BlueGnome Ltd., Cambridge, United Kingdom). Microarray data was processed and normalized using JMP Genomics (SAS Institute Inc., Cary, NC). Expressed probes were identified as those whose median signal intensity was brighter than the 99th percentile of negative control expression values. Significance of gene expression was determined by ANOVA. P-values were adjusted for a false discovery rate controlled P-value threshold of 0.05. The results from expression arrays confirmed expression of clock genes in PMM DA-MEL neurons. The upregulation of *Per3*, *Cry2* and *Bmal1* was observed in SD birds (CT14, dark phase). In contrast, *Cry1*, *Per2*, *Bmal2* and *Clock* were upregulated in LD birds (CT14, light phase). In addition, expression of photopigment molecules was observed in PMM DA-MEL neurons including rhodopsin, panopsin and melanopsin. Upregulation of the rhodopsin gene was also observed in LD birds, as compared with SD birds, along with the genes encoding rod cGMP-specific phosphodiesterase 6B (PDE6B) and retinal pigment epithelium-specific protein (RPE65). This interesting expression shift following photostimulation implies possible light perception. The expression of photopigments, signaling molecules, and clock genes in PMM DA-MEL neurons provides additional support for this hypothalamic region as a site of photoreception and photoperiodic time measurement.

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Key Words: birds, circadian rhythm, photoreception

708 Septal and hypothalamic structures activated following sexual and agonistic encounters in male broiler breeders. W. J. Kuenzel*, J. Xie, and A. Jurkevich, *University of Arkansas*, *Fayetteville*.

Induction of Fos protein, an indicator of neuronal activation, was utilized to identify groups of neurons activated by either sexual or aggressive interactions in roosters. Experimental groups included handled controls, non-contact interaction with a female (M-FN), or contact interaction with a taxidermy female (M-FT), a live female (M-FC) or a live male (M-M). Eight brain areas were examined and 6 will be discussed. Results showed that the medial portion of the bed nucleus of stria terminalis, subnucleus 2 (BSTM2) was activated solely by appetitive sexual behavior (M-FN). Consummatory sexual behavior (M-FT and M-FC) resulted in significantly higher Fos protein counts in the medial preoptic nucleus (POM), lateral septum (SL), paraventricular nucleus (PVN),ventral lateral thalamic area (VLT) and bed nucleus of the pallial commissure (NCPa). Aggressive behavior (M-M) resulted in activation of the POM, SL, PVN, VLT and NCPa. It is seen that 5 neural structures were activated for both sexual and aggressive behavior suggesting that the same neural structures are utilized for functionally distinct behaviors. The most pronounced increase in Fos counts due to agonistic behavior was seen in the PVN and dual immunocytochemical studies showed that induced Fos protein occurred in magnocellular arginine vasotocin (AVT) neurons. Since magnocellular AVT neurons project directly to the posterior pituitary, it suggests that the stress response depends upon peripheral release of AVT independent of the established peptide release into the median eminence and portal system for activating ACTH secretion from the anterior pituitary. In summary, results demonstrate that use of Fos protein is effective for elucidating differential sets of neurons involved in specific behaviors of chickens.

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Key Words: Fos protein, bed nucleus of stria terminalis, arginine vasotocin

709 Various social behaviors induce differential activation of aromatase neurons in the brain of male broilers. J. Xie*, W. J. Kuenzel, and A. Jurkevich, *University of Arkansas*, *Fayetteville*.

Several steroid-sensitive nuclei in the brain form a network regulating various social behaviors. The medial preoptic area (POM) and the medial portion of bed nucleus of stria terminalis (BSTM) are important components of this network. Our previous study demonstrated that the POM is activated following male sexual behavior and intermale conflict. The subnucleus 2 of BSTM (BSTM2) was specifically activated by appetitive sexual behavior. The objective of this study was to investigate the activation of aromatase-immunoreactive (ARO-ir) neurons in the POM and BSTM using dual immunolabeling for ARO and immediate early gene product, Fos. Males were subjected to a 20-min non-contact interaction with a female (M-FN), or contact interaction with a female (M-FC) or another male (M-M). Handling (HC) and open-field (OF) groups were used as controls. In the POM, intermale interactions decreased the total number of ARO-ir cells, while M-FN had more ARO+Fos-ir neurons than other groups. The lateral portion of POM had more ARO+Fos-ir cells than the medial portion. In the BSTM1, M-FN had a higher percentage of ARO+Fos-ir cells than HC and M-M groups. In the BSTM2, social interactions resulted in a decrease of ARO-ir cells with lowest number of ir cells in the M-FC group. M-FN had significantly more ARO+Fos-ir cells than any other treatment group. In the lateral POM, ARO+Fos-ir cells positively correlated with the frequency of waltzing toward a female (courtship), while waltzing toward a male (agonistic display) negatively correlated with ARO-ir cell counts in the medial POM. In the BSTM1 and 2, positive correlations were found between ARO+Fos colocalization and waltzing toward a female, while chasing and waltzing frequency in intermale interactions negatively correlated with ARO-ir cell counts in the BSTM1 and 2, respectively. The findings suggest that activation of different ARO-ir cell groups may underlay the roles of POM and BSTM in distinct behaviors.

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Key Words: neuroendocrine regulation, mating, aggression

710 Fos protein induction in vasotocinergic neurons of male broilers following different social contexts. A. Jurkevich*, J. Xie, and W. J. Kuenzel, *University of Arkansas*, *Fayetteville*.

Vasotocin (VT) in avian species and its mammalian counterpart vasopressin are important peptidergic regulators of social behavior. The medial portion of the bed nucleus of stria terminalis (BSTM) of chickens contains parvocellular neurons producing VT in a sex dimorphic pattern with males having abundant VT cells and projections and females virtually devoid of VT in this location. In males, these cells also express galanin, corticotropin-releasing hormone and estradiol-producing enzyme aromatase suggesting important roles of this relatively small neural system in neuroendocrine regulation. The aim of this study was to reveal vasotocinergic neurons activated in roosters following different social encounters using Fos protein as an indicator of metabolic stimulation. Individual roosters were placed in an observation pen where they were provided with restricted or unrestricted access to a female (groups M-FN and M-FC), unrestricted access to a male (group M-M) or were exposed

to an empty pen (open field control, OF). After completion of tests, neurons immunolabeled for VT and Fos were quantified in 2 portions of the BSTM. In the dorsolateral subnucleus of the BSTM (BSTM1), male-female or male-male interactions did not change the total number of VT cells or percentage of VT cells co-expressing Fos. In the ventromedial subnucleus of the BSTM (BSTM2), there was a significant increase ($P < 0.05$) in percentage of VT cells co-expressing Fos in males that had non-contact interactions with females (group M-FN, 49.2 ± 4.8) as compared with control males exposed to an empty pen (group OF, 29.4 ± 7.1). Same-sex interactions resulted in lower percentage of VT and Fos co-expressing cells in BSTM2 than opposite-sex interactions ($P < 0.05$). The findings demonstrate that vasotocinergic neurons in BSTM2 of males are preferentially activated following opposite-sex interactions and confirm our previous observations regarding a key role of BSTM2 in control of male sexual behavior in roosters.

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Key Words: broiler breeders, mating, aggression

711 Effects of RFamide-related peptide-3 (RFRP-3) on secretion of LH in ovariectomized prepubertal gilts. N. L. Heidorn[1], C. R. Barb[2], C. J. Rogers[1], G. J. Hausman[2], and C. A. Lents*[1], [1]*University of Georgia, Athens,* [2]*USDA-ARS Richard B. Russell Agriculture Research Center, Athens, GA*.

Pulses of LH are suppressed before puberty in the gilt. RFRP-3 is proposed to be a hypophysiotropic hormone in mammals. A series of experiments (EXP) were conducted to test the hypothesis that RFRP-3 inhibits LH release in ovariectomized (OVX) prepubertal gilts. All gilts were OVX at least 2 weeks before being fitted with indwelling jugular catheters for the collection of serial blood samples. In EXP I, blood samples were collected every 15 min for 6 h. Commencing at 120 min after the start of sampling, all gilts (n = 3) received a loading dose of 1 mg of RFRP-3 followed by repeated injections of 40 µg of RFRP-3 every 5 min for 2 h resulting in a total infusion of 2 mg of RFRP-3. All injections were administered by hand in 2 mL of 0.9% saline. Area under the curve (AUC) was determined in each of 3 periods; 1 h before treatment (period 1), the first h of treatment (period 2), and the second h of treatment (period 3). In EXP II, blood samples were collected every 15 min for 8 h. Commencing at 240 min after the start of sampling, animals received intracerebroventricular (i.c.v.) injections of 10, 50, or 100 µg of RFRP-3 in 0.9% saline (n = 6/group). Control animals received 0.9% saline alone (n = 7). AUC was determined for each of 2 periods (4 h before and 4 h after i.c.v. treatment). Mean LH (1.33 ± 0.13 ng/mL), number of LH pulses (2.0), or pulse amplitude (1.32 ± 0.25 ng/mL) was not different during the 2-h treatment period when compared with 2 h pre- or the 2 h post-treatment. However, there was a tendency ($P = 0.09$) for total LH release, as indicated by AUC, to be reduced in period 3 compared with periods 1 or 2. In EXP II, central administration of 10 µg of RFRP-3 yielded an apparent suppression in area under the curve (59.4 ± 20.91% of the pre-i.c.v. value), but this did not reach significance ($P = 0.27$). We conclude that RFRP-3 does not act to inhibit the pulsatile release of LH in prepubertal gilts.

Key Words: GnIH, RFRP-3, LH

712 The effects of fluoxetine on lactation and lamb growth in sheep. P. L. Black*[1], R. A. Halalsheh[1], L. M. Lankford[1], M. M. Marricle[1], M. M. Christiansen[1], M. M. Scroppo[1], L. L. Hernandez[2], and T. T. Ross[1], [1]*New Mexico State University, Las Cruces,* [2]*University of Cincinnati, Cincinnati, OH*.

Fluoxetine (a selective serotonin reuptake inhibitor; FLX) has been shown to cause a delay in the onset of lactogenesis stage II when taken during pregnancy and/or lactation. A study was conducted to evaluate if ewes would be an appropriate model to determine the effects of FLX on milk production. Twenty-nine ewes (85 ± 12 kg; body condition score 2.6 ± 0.3) in late gestation were used in this study. Ewes allotted to treatments were stratified by fetal numbers and breeding date. Ewes were orally dosed daily with an empty capsule for controls or a capsule containing 40 mg of FLX. Dosing began on about d 121 of gestation and continued until lambing. Ewes were dosed every morning at 0700 h. Following parturition and before nursing, milk and blood samples were collected from each ewe and her lamb(s). The first milk yield was measured 8 h after birth and subsequent milkings were conducted at 1500 and 1800 h every other day for 9 d. Milk letdown was induced by a 1 mL intravenous injection of oxytocin, 1 min before milking. Milk yields were measured over a 3 h period when lamb(s) were removed. We observed a treatment by parity interaction, as ewes with multiple lambs treated with FLX had greater ($P = 0.01$) milk yields than treated or control ewes giving birth to single lambs and control ewes giving birth to multiple lambs. Lambs were weighed at birth (d 0) and following the milk yield study (d 9). We observed no differences ($P > 0.05$) in either birth weight or d 9 weights. Lamb gain over the 9 d milking period was similar among treated and control ewes ($P > 0.05$). No interactions were observed between parity and treatment in lamb weights or gain. Fluoxetine treatment during late pregnancy resulted in greater milk production in ewes giving birth to multiple lambs. However, FLX had no effect on lamb weights or lamb weight gain.

Key Words: fluoxetine, lactation, sheep

714 Cloning and characterization of chicken galanin and galanin receptors. J. C. W. Ho*[1], Y Wang[2], and F. C. Leung[1], [1]*The University of Hong Kong, Hong Kong, HKSAR, China,* [2]*Sichuan University, Chengdu, Sichuan, China*.

Galanin is a neuropeptide of 29 to 30 amino acids, widely distributed in the mammalian nervous systems and peripheral tissues. It exerts multiple physiological functions including modulation of cognitive functions and hormones release through the interaction with at least 3 known G protein-coupled receptors (GalR1, 2 and 3), which have only been identified in mammals. In the present study, 4 transcript variants of galanin prepropeptide precursor (cGAL), 3 galanin receptors (cGalR1, 2 and 3) and 2 additional receptors with considerable homology to cGalR1 and cGalR2, thus herein designated cGalR1-L and cGalR2-L, were cloned from chicken whole brain and intestine tissue respectively. Four variant cDNAs for chicken galanin prepropeptide, resulted from alternative splicing, encode precursor peptides of 88, 117, 141 and 150 amino acids respectively, while the 5 cloned receptors are ranged from 357 to 405 amino acids in lengths, sharing considerable amino acid sequence identities (50% to 86%) to their mammalian homologs. Using reverse transcription-polymerase chain reaction (RT-PCR), cGAL and its receptors were found to be widely distributed in the 12 adult chicken tissues and different regions of oviduct examined, with particularly high abundances in brain, small intestine, ovary and pituitary, except for cGalR3 in which its expression was restricted to ovary. Using different luciferase reporter systems, we also demonstrated that chicken galanin peptide was capable of altering luciferase activities, in dose-depending manners, in Chinese hamster ovary (CHO) cells expressing each cloned receptor, thus suggesting the differential functional couplings of each receptor to various classes of G proteins. The characterization of chicken galanin receptors would provide a better understanding to the physiological functions of galanin in avian species.

Key Words: chicken, galanin, galanin receptor