Physiology: Physiology III

171 Distribution of serotonergic neurons and fibers and interaction with dopaminergic neurons in the turkey hypothalamus and brainstem. S. Kang*, O. Youngren, T. Bakken, and M. El Halawani, *University of Minnesota, St. Paul*.

Serotonin (5-HT) is involved in avain prolactin (PRL) secretion through the dopaminergic system. However, little is known about the neuroanatomical association of 5-HT with dopamine (DA) neurons in the hypothalamus and brainstem. To investigate the relationship of DA and 5-HT in the avian reproductive neural system, the distribution of 5-HT-synthesizing neurons and fibers, and the interaction of 5-HT with DA neurons were examined in the adult female turkey brain. The localization and the association of 5-HT and DA neurons and fibers were studied using double immunocytochemistry with 5-HT and tyrosine hydroxylase (TH) antibodies. 5-HT-immunoreactive (ir) neurons and fibers were located in the hypothalamic paraventricular organ (PVO), and in the brainstem, where there were three separate cell groups: the ventro-rostral mesencephalon group, the dorsal mesencephalo-pontine group, and ventro-pontine group. 5-HT-ir fibers were widely distributed throughout the turkey hypothalamus, and were particularly abundant in the septum lateralis (SL), nucleus preopticus medialis (POM), nucleus anterior medialis hypothalami (AM), nucleus suprachiasmaticus pars medialis (SCNm), nucleus commissurae pallii (nCPa), nucleus mamillaris medialis (MM), nucleus mamillaris lateralis (ML) and the external zone of the median eminence (ME). TH-ir cell bodies in the SL, POM, SCNm, AM, MM, ML, and the area ventralis of Tsai (AVT) were intermingled with 5-HT fibers. The distribution of 5-HT ir neurons and fibers observed in this study confirm and extend results previously reported in the brain of other avian species. These data suggest that the serotonergic neural system in the turkey hypothalamus has close neuroanatomical association with the DA neurons which may be involved in PRL secretion. USDA grant No. 2004-35203-14771

Key Words: Serotonin (5-HT) neuron, Turkey hypothalamus and brainstem, Dopamine

172 The role of retinal and extra-retinal photoreceptors in reproductive activities of broiler breeder hens. I. Rozenboim*, N. Mobarkey, O. Oshpiz, N. Avital, and R. Heiblum, *Hebrew University of Jerusalem, Rehovot, Israel*.

Photostimulation plays an important role in reproductive activities of birds. Light quality can be determined by: 1. Photoperiod, 2. Intensity and 3. Spectral energy distribution. Photostimulation in birds can be received by retinal and extra retinal photoreceptors which are located in the pineal gland, hypothalamus, pituitary and olfactory bulbs. Long wave length radiation (red wave) can be characterized by deep tissue penetration affecting hypothalamic function. In contrast, activation of retinal photoreceptors by green-yellow waves appears to be inhibitory to reproduction.

The purpose of this study was to investigate the interaction of retinal and extra retinal photoreceptors on reproductive activities of broiler breeder hens.

Animals: 130 broiler breeder hens at 23 weeks of age were divided into nine environmental and light controlled rooms. Hens were reared in battery. Three rooms were photostimulated (14L:10D) with full light spectrum (white light; 29 lux), and served as control. Six rooms were equipped with two lighting systems; red (0.16 Watt) and green (27.5 lux). Upon photostimulation three rooms were photostimulated by increasing red light to 14 hours of light (green light remained of six hour) and the last three rooms were photostimulated by increasing green light to 14 hours of light (red light remained of 6 hours). Monochromatic lights were provided by LED laps located at the top of the cage. Egg production, as well as ovarian steroid levels were measured. Photostimulation with green light caused a significant delay in the reproductive activities manifested by low egg production and plasma gonadal steroid levels during the 12 weeks post photostimulation. Green photostimulated birds had lower cumulative egg production during all experimental period. Hens photostimulated with red light had higher egg production compared to the rest of the groups. It seems that retinal photostimulation delays, whereas extra-retinal photostimulation accelerates reproductive activities of broiler breeder hens.

Key Words: Retinal, Broiler hens, Photoreceptors

173 Cloning of Smad 1, Smad5 and Smad8 from the chicken ovary and characterization of their expression during chicken ovarian development. J. Li*, Y-J. Wang, C-C. Hon, and F. C. Leung, *The University of Hong Kong, Hong Kong, HK-SAR, China*.

We have previously demonstrated that the oocyte-secreted bone morphogenetic protein 15 (BMP15) and its cognate receptors are abundantly expressed in the ovaries from both sexual immature and mature chickens, strongly suggesting that it plays an important role in controlling ovarian development. To test this hypothesis, in the present study, we have further examined the expression of the downstream signaling molecules (Smad1, Smad5 and Smad8) responsible for BMP15 signaling in the chicken ovary. We first cloned the full-length cDNAs encoding Smad1, Smad5 and Smad8 from the chicken ovary. The cloned Smad1 (465 a. a.), Smad5 (465 a. a.) and Smad8 (476 a. a.) share high homologies with their mammalian counterparts (Smad1: ~96%, Smad5: ~95%, Smad8: ~80%). Using semi-quantitative RT-PCR, we demonstrated that Smad1, Smad5 and Smad8 were expressed not only in all of the developing follicles from adult chickens, but also in the developing ovaries of all stages examined (3-week, 6-week, 10-week, 13-week and 16-week). Moreover, the expression of Smad1, Smad5 and Smad8 could also be detected in the cultured ovarian granulosa cells from F4+5 and F1 follicles, suggesting that the ovarian granulosa cell is one of the action sites of oocyte-derived BMP15. Taken together, the co-expression of BMP15, BMP15 receptor and its downstream signaling molecules (Smad1, 5 and Smad8) in the chicken ovary provides convincing evidence that BMP15 is actively involved in controlling ovarian development in chicken.

Key Words: Smad, BMP15, Ovarian Development

174 Cloning of chicken pre-B-cell colony-enhancing factor 1 (PBEF1) and characterization of its spatial expression. J. Li*, Y-J Wang, and F. C. Leung, *The University of Hong Kong, Hong Kong, HK-SAR, China*.

The pre-B-cell colony-enhancing factor 1 (PBEF1) was originally identified from lymphocytes in human as a cytokine to enhance the pre-B-cell colony formation. However, most recent studies demonstrated that it could also mimic effects of insulin and lower plasma glucose level in mice. Using chicken as the experiment model to explore its potential physiological roles in lower vertebrates, we cloned the full-length cDNA coding for PBEF1 from chicken ovary. It encodes for a 493 amino acid peptide and shares extremely high homology (~94%) with that of mouse, rat and human. Interestingly, unlike most of the cytokines identified, the putative chicken PBEF1 lacks a typical signal peptide sequence at its N-terminus, thereby raising an interesting issue whether Chicken PBEF1 represents a secreted cytokine or a cytosolic enzyme as reported in rats and mice. Using RT-PCR and Northern blot analysis, we noticed that chicken PBEF1 was abundantly expressed in all of the examined tissues including brain, heart, lung, liver, intestine, kidney, muscle, ovary, pancreas, testis, pituitary and spleen. The ubiquitous expression of chicken PBEF1, together with its conserved amino acid sequence, clearly implies that PBEF1 might play critical role in a variety of tissues besides lymphoid tissue and fatty tissues found in vertebrates.

Key Words: PBEF1, Chicken, Cytokine

175 Development of the small intestinal epithelial barrier function in broiler chicks. S. Roberts*, M. Perez-Garcia, M. Neal, and K. Bregendahl, *Iowa State University, Ames*.

The small intestines (SI) of broiler chicks are not fully developed at hatch, taking 10-14 days before nutrient digestibility is maximized. During this time, marked changes in small intestinal morphology are observed as well, suggesting that small intestinal epithelial barrier function also develops after hatching. Male 1-d-old Ross-308 broiler chicks were housed in batteries with free access to a common diet and water. On each of d 2, 3, 4, 5, 6, 7, 10, 13, and 14 of age, 8 chicks were randomly selected and euthanized. The jejunum and ileum were removed and mid-sections mounted in Ussing chambers containing oxygenated Ringer's solution. Voltage and current electrodes were connected to a cur-

rent clamp, and a 40-μA current was applied after 20 min. The resultant change in voltage across the tissue was recorded and the transepithelial electrical resistance (TER) calculated according to Ohm's law. The TER measures paracellular permeability through tight junctions and indicates the SI's ability to exclude luminal antigens. Another 3 chicks were euthanized each day for measurement of jejunal and ileal mRNA levels of the tight-junction proteins, occludin and zonula occludens-2, using real-time PCR. Data were analyzed by broken-line and linear regressions using JMP. The jejunal TER increased from 65±12 Ω cm^2 (mean±SEM, n=8) at d 2 of age and reached a plateau of 288±15 Ω cm^2 at d 11 of age ($P<0.05$). The ileal TER increased linearly from 42±5 Ω cm^2 at d 2 to 95±9 Ω cm^2 at d 14 of age ($P<0.01$), but did not reach a plateau ($P>0.05$). Jejunal occludin expression increased linearly with age ($P<0.01$), but did not reach a plateau ($P>0.05$). No effects of age on ileal occludin or on zonula occludens-2 expression were observed ($P>0.05$). The results show that the epithelial barrier function of the SI is not fully developed in broiler chicks until d 11 of age for the jejunum and later than d 14 of age for the ileum. Further experiments are warranted to investigate the correlation between the change in small intestinal TER with age and the expression, subcellular location, and amount of tight-junction proteins.

Key Words: Intestinal development, Transepithelial electrical resistance, Tight junction mRNA expression

176 Role of glutamate receptor in food intake regulation in chickens.

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Previous research has shown that intracerebroventricular (ICV) injections of glutamate decreased food intake in broilers and Leghorns. This effect appeared to be mediated by both 1,2-amino-4-phosphonobutyrate (AMPA) and N-methyl-D-aspartate (NMDA) glutamate receptors. The present study was designed to further investigate the role of the NMDA receptor in food intake regulation in chickens. Glycine and D-cycloserine can modify the action of the NMDA receptor. Cycloserine acts as a partial agonist of the NMDA receptor. While under sodium pentobarbital anesthesia, a 23-gauge thin-walled stainless steel cannula was stereotaxically implanted into the right lateral ventricle of 3 or 8 week old male broilers and Leghorns, respectively. The ICV injection of cycloserine caused a dose-dependent decrease in food intake of male broilers and Leghorns. The ICV injectioni of glycine also caused a dose-dependent decrease in food intake of broilers, but not Leghorns. Additional experiments were conducted to determine the affect of oral administration of these compounds on food intake. Oral glycine decreased food intake in both male and female broilers whereas oral glycine decreased food intake in female, but not male, Leghorns. These results further support a role for the NMDA receptor in food intake regulation in chickens.

Key Words: Food intake, NMDA receptor, Chickens