Control of the reproductive axis: Balancing act between stimulatory and inhibitory inputs

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ABSTRACT As for most vertebrates, reproduction in poultry is controlled by an integrated axis [the hypothalamo-pituitary-gonadal (HPG) axis]. External and internal cues are integrated at the level of the hypothalamus to initiate gonadal recruitment and control the subsequent reproductive cycle. Until recently, it was believed that the HPG was exclusively under stimulatory control from hypothalamic gonadotropin releasing hormone (GnRH). However in 2000, the discovery of gonadotropin inhibitory hormone (GnIH), an inhibitory peptide, changed this dogma. Since then, evidence accumulated to confirm that in fact the HPG is under a dual control system with a stimulatory and an inhibitory branch. In this paper, we review the organization of this dual control system, the mechanisms controlling the synthesis and release of GnRH and GnIH, and the possible integration and interactions between the two branches to regulate pituitary gonadotropes’ function. Furthermore, as light perception and photoperiod are the primary cues utilized by the poultry industry in controlled environments, special consideration was given to potential practical applications.

Key words: reproduction, gonadotropin releasing hormone, gonadotropin inhibitory hormone, lighting, photoperiod

OVERVIEW OF THE REPRODUCTIVE AXIS

Reproduction in poultry is controlled by a neuroendocrine axis comprised of the hypothalamus, the anterior pituitary gland, and the gonads [i.e., the hypothalamo-pituitary-gonadal (HPG) axis]. The hypothalamus serves as an integration center and coordinates the activation and inhibition of the axis by releasing neuropeptides in the portal vascular system. In turn, upon binding to their cognate-specific receptors on pituitary gonadotropes, these peptides control the synthesis and release of gonadotropins [luteinizing hormone (LH) and follicle stimulating hormone (FSH)] into the systemic circulation. Both LH and FSH act at the level of the gonads to initiate sexual maturation, by stimulating gametogenesis and the synthesis of sex steroid hormones. Like most endocrine axes, the HPG is primarily under feedback control.

At the level of the hypothalamus, stimulatory and inhibitory neuropeptides correspond to gonadotropin releasing hormones (GnRHs) and gonadotropin inhibitory hormone (GnIH), respectively. In chickens, two GnRHs have been characterized [chicken cGnRH-I (cGnRH-1) and cGnRH-II] (Miyamoto et al., 1982; Miyamoto et al., 1983; Miyamoto et al., 1984), and although both GnRHs have the ability to stimulate the production of gonadadotropins (Hattori et al., 1986), it is well-established that cGnRH-I is the hypophysiotropic peptide. This is supported by the fact that cGnRH-I perikarya are mainly located in the septal/preoptic hypothalamus projecting to the median eminence (ME), while cGnRH-II perikarya are mainly located around the occulomotor complex extending to the mesencephalon (Mikami et al., 1988; Kuenzel and Blahser, 1991; Van Gils et al., 1993), and immunization against cGnRH-I (not cGnRH-II) induces gonadal reduction in laying hens (Sharp et al., 1990). On the other hand, GnIH, a peptide from the RF-amide [carboxyterminal arginine (R) with an amidated phenylalanine (F) motif] family first isolated from quail brain (Tsutsui et al., 2000), was shown to inhibit LH synthesis and release (Ciccone et al., 2004; Ubuka et al., 2006). GnIH perikarya are located in the paraventricular nucleus (PVN) with fibers contacting GnRH-I and GnRH-II neurons, suggesting a direct effect on GnRH synthesis/release (Satake et al., 2001, Bentley et al., 2003, Ukena et al., 2003; Osugi et al., 2004). Furthermore, nerve terminals are also present in the ME, indicating GnIH release into the portal vascular system to directly inhibit pituitary gonadotropes’ function (Tsutsui et al., 2000).

In chickens, two GnRH receptors (GnRHRs) have been characterized, with cGnRHR-I expressed in multiple tissues (including the brain, pituitary, and gonads) (Sun et al., 2000), and cGnRHR-2 being pituitary-specific (Shimizu and Bedecarrats, 2006). Based on...
phylogenetic analyses, subsequent studies reclassified cGnRHR-2 as a type III receptor and confirm that its messenger RNA (mRNA) levels are 1,400-fold more abundant than cGnRHR-I (Joseph et al., 2009). Thus, to avoid any confusion, we will refer to this receptor as cGnRHR-III. Both receptors are G-protein coupled receptors and have been shown to couple not only to Gq alpha subunit, leading to an increase in intracellular inositol phosphates (Sun et al., 2001; Shimizu and Bedecarrats, 2006), but also to Gs alpha subunit, resulting in the activation of cyclic adenosine monophosphate (cAMP) pathway (Shimizu and Bedecarrats, 2010).

The receptor for GnIH (i.e., GnIHR) was first characterized in quail (Yin et al., 2005) and shown to be widely expressed in the brain, pituitary, and reproductive organs. In chickens, two potential receptors were reported, with one displaying higher affinity to GnIH (Iketomo and Park, 2005). This receptor was later shown to be expressed in the pituitary gland on both LH- and FSH-producing cells (Maddineni et al., 2008), reinforcing the idea of a direct action of GnIH on gonadotropes. Similarly to cGnRHRs, cGnIHR is a G-protein coupled receptor. However, we showed that it couples to Gi alpha subunit, thereby inhibiting the activity of adenyl cyclase and the production of intracellular cAMP (Shimizu and Bedecarrats, 2010).

### CONTROL AND INTEGRATION OF STIMULATORY AND INHIBITORY INPUTS

As seasonal breeders, chickens have the ability to utilize external environmental cues to initiate and terminate reproduction. Under temperate latitudes, photoperiod is the predominant indicator with increase in day length resulting in gonadal recruitment. This has been extensively utilized by the poultry industry to manage and maximize reproduction under controlled environments. Although it has been well-established that an increase in photoperiod beyond 12 h can stimulate the synthesis and release of GnRH by the hypothalamus (Dunn and Sharp, 1990), the exact mechanisms involved in the transduction of light energy into a neuroendocrine signal is still not fully understood. However, evidence point to direct detection by deep brain photoreceptors located within the hypothalamus (Sharp, 1993; Saldanha et al., 2001). The possible location and mode of action of such photoreceptors is reviewed in this issue by Kuenzel as part of this symposium, and may involve the action of TSH and thyroid hormones as previously reported (Yoshimura et al., 2003; Nakane and Yoshimura, 2014). On the other hand, experimental evidence shows that the synthesis and release of GnIH is primarily under the influence of melatonin, produced by both the pineal gland and the retina of the eye. This was first reported by Ubuka et al. (2005) who showed that the melatonin receptor Mel1c is present on GnIH neurons in quail, and removal of endogenous melatonin sources (pinealectomy and enucleation) reduces levels of GnIH mRNA and peptide within the hypothalamus. Furthermore, this effect was reversed when exogenous melatonin was administered. More recently, it has also been shown that melatonin can stimulate the release of GnIH by hypothalamic explants (Chowdhury et al., 2010), and in vivo melatonin and GnIH follow similar diurnal patterns (Chowdhury et al., 2010; 2013). As a matter of fact, the effect of melatonin may not be limited to birds and may be conserved amongst multiple seasonal breeders including mammals (Tsutsui et al., 2013). Thus, under short days, melatonin produced by the pineal gland and retina of the eye is at its highest, stimulating the release of GnIH and maintaining an inhibition on the HPG axis. As day length increases, reduced melatonin production lifts GnIH inhibition, while this increase in exposure to light directly stimulates hypothalamic photoreceptors, leading to the activation of the stimulatory pathway via GnRH. Under this model, the hypothalamus modulates pituitary gonadotropes’ function by changing the ratio of inhibitory (GnIH) versus stimulatory (GnRH-I) neuropeptides released into the portal vascular system.

At the level of the pituitary gland, we have also shown that the sensitivity to hypothalamic neuropeptides switches around the time of photostimulation. In both males and females, levels of cGnRHR-III mRNA are lowest in immature birds, highest postphotostimulation, and progressively decrease towards the end of an active reproductive period (Shimizu and Bedecarrats, 2006). Conversely in the same pituitary samples, levels of cGnIHR mRNA are the highest in immature birds and lowest postphotostimulation (Shimizu and Bedecarrats, 2010). This suggests that the activation/inhibition of the HPG is not only under the control of the ratio of hypothalamic neuropeptides, but also possibly regulated at the levels of the anterior pituitary gland by changing the ratio of receptors. We thus further investigated the possible consequence of such a switch in receptor ratio in vitro. As mentioned above, cGnIHR couples to Gi to inhibit adenyl cyclase and reduce intracellular cAMP production, while activation of cGnRHR-III can lead to an increase in cAMP mediated response. In cells cotransfected with both cGnRHR-III and cGnIHR, cGnIHR was able to significantly reduce cGnRH-I-induced cAMP response in a dose-dependent manner (Shimizu and Bedecarrats, 2010); however, the inhibition was not complete, suggesting that part of the GnRH induced cAMP response is independent of adenyl cyclase and may involve cross talk between the Gq and Gs pathways. Furthermore, we also showed that the blocking effect of cGnIH on cGnRH-I action is dependent on the ratio of receptor present (Shimizu and Bedecarrats, 2010). These results suggest that GnIH and GnRH regulate pituitary gonadotropes’ function by interacting at the intracellular signaling pathway level. However, such a model would be valid if both receptors are present on the same cells in vivo. To test this hypothesis, we performed
immunohistochemistry analyses on anterior pituitary slices using antibodies generated specifically against cGnRHR-III (McFarlane et al., 2011) and cGnIHR (Maddineni et al., 2008). Results showed that cGnRHR-III immunoreactive cells are scattered throughout both cephalic and caudal lobes, but are more concentrated in the edges of the cephalic lobe in an area where cGnIHR is present. Unfortunately, both antibodies were raised in rabbits, which prevented colocalization; however, immunohistology of subsequent slices showed that positive cells were overlapping strongly, suggesting that indeed both receptors are present on the same cells.

Taken together, these results led us to propose a model describing the interaction of cGnRHR-III and cGnIHR, to integrate both stimulatory and inhibitory hypothalamic inputs at the level of the pituitary gland (Bédécarrats et al., 2009). Nonetheless, in this model, one question remains: what controls the switch in receptor expression? Evidence suggests that steroid feedback from the gonads may be a key component, as injection of estradiol (or a combination of estradiol and progesterone) decreases pituitary mRNA levels of cGnIHR in immature chickens (Maddineni et al., 2008). In addition, whether cGnIHR and cGnRH-I control the gene expression of their own receptors may also be a possibility that needs to be investigated.

IMPACT OF PHOTORECEPTION

As mentioned above, the key to integrate photoperiod is the ability to transduce light into neuroendocrine signals. This implies specific photoreceptors on cells that can directly or indirectly regulate the synthesis of GnRHs and GnIH. In birds, the three main photosensitive organs correspond to the retina of the eye, the pineal gland, and deep brain (hypothalamic) photoreceptors. The relative contribution of each organ in the control of the HPG has been somewhat controversial, although it was shown that direct photostimulation of the hypothalamus is sufficient (Benoit, 1964; Saldanha et al., 2001), while removal of the eye did not impact sexual maturation (Salter et al., 1997). Retinal cell loss in these birds occurs early on during development (Tran et al., 2013) and by 6 wk age, homozygous individuals are totally blind. After maintaining several generations, we noticed that both males and females displayed advanced sexual maturation. Thus, we opted to use this line as a model to study the effect of the retina on the HPG. When left under a nonstimulatory photoperiod, testicular growth and spermatogenesis in blind males was spontaneously initiated earlier than their sighted counterparts (Perttula and Bédécarrats, 2012). This suggests that the retina of the eye may have in fact an inhibitory input on the HPG. We thus speculated that melatonin produced by the retina under short days stimulated GnIH production, maintaining a “break” on hypothalamic GnRH and the production of gonadotropin by the pituitary gland. However, recent results (unpublished data) show that the lack of a functional retina in blind birds does not significantly impact melatonin pineal content or plasma levels, although levels in the retina of the eye were drastically reduced. Furthermore, GnIH concentration in the diencephalon was not different between blind and sighted animals. Thus, conversely to previous reports in quail (Ubuca et al., 2005; Chowdhury et al., 2010; 2013), the lack of melatonin from the retina in Smoky Joe roosters did not impact systemic levels and did not reduce GnIH levels in the diencephalon, suggesting the early sexual maturation observed in these birds is not caused by reduced inhibitory input from the hypothalamus as we previously hypothesized. However, whether the lack of retinal input affected the stimulatory branch (GnRH) was not investigated and further studies will be required to confirm these observations.

SPECTRUM LIGHTING

One key component of any photosensitive organ is the density and type of photoreceptors present. In chickens, the retina of the eye possesses four types of cones sensitive to either, red, green, blue, or UV light, with the peak sensitivity in the green spectrum (Prescott and Wathes, 1999). On the other hand, the pineal gland possesses a specific opsin called “pinopsin,” which appear to be more sensitive to intensity rather than wavelength (Pang et al., 1974; Nir et al., 1987). At the level of the hypothalamus, red opsin appears to be predominant in boiler breeders and red light is more effective in activating the HPG (Mobarkey et al., 2010). Thus, the quality of light (spectral output) may be more important than the photoperiod itself. We recently reported that using our blind line of Smoky Joe chickens, red light was required to mediate the activation of the HPG resulting in highest levels of estradiol, while green light was ineffective (Baxter et al., 2014). Interestingly, although no significant differences in age at first egg were observed between blind and sighted birds for each light treatment, under green light, sighted birds dropped out of production earlier (Baxter et al., 2014). Whether green light stimulated the inhibitory branch via the melatonin/GnIH system, or just failed to activate the stimulatory branch via GnRH, was not investigated and further studies are underway. Nonetheless, our results clearly show that red light is required to adequately stimulate the activation of the HPG.

When switching from our experimental Smoky Joe model to commercial lines of layers, we also observed a greater stimulation of the HPG under red light, as
indicated by increased estradiol level during sexual maturation (Baxter and Bédécarrats, 2014). However, no significant difference in overall production was observed. Interestingly, hens from these commercial lines tended to spontaneously mature before photostimulation, suggesting that beyond the photoperiod, modern layers may rely on other cues to initiate reproduction. One such cue may be metabolic and related to body weight. Interestingly, GnIH has also been shown to stimulate feeding behavior in chicks (Tachibana et al., 2005). Thus, it is conceivable that all external and internal cues converge toward a central integration center, which in turn regulates GnRH synthesis and release. In mammals, this integration center has been characterized as the kisspeptin/neurokinin B/dynorphin (KNDy) neuronal network. However, it has become clear that the kiss genes (kiss1 and kiss2) are absent from the chicken genome and they may have been lost during evolution (Joseph et al., 2013; Pasquier et al., 2014), and whether an equivalent system exists remains to be determined.

CONCLUSION

From the first report of GnIH and its role as an inhibitory neuropeptide on the HPG (Tsutsui et al., 2000), it has been clearly established that the hypothalamus utilizes a dual control (stimulatory/inhibitory) system to regulate reproductive function in chickens (Figure 1), and that external and internal cues are integrated, to modulate the production and release of GnIH and GnRH. Under short days, melatonin produced by the retina of the eye and the pineal gland maintains high levels of cGnIH, which prevents the release of cGnRH-II from the hypothalamus and inhibits GnRH action on pituitary gonadotropes, thus preventing the activation

**Figure 1.** Summary of the dual stimulatory/inhibitory control of reproduction in chickens; A = under short days, the pineal gland and retina of the eyes both increase melatonin (MEL) production, which in turn activates the hypothalamic synthesis and release of chicken gonadotropin inhibitory hormone (cGnIH). Increased cGnIH activity directly inhibits chicken gonadotropin releasing hormone I (cGnRH-I) neurones within the hypothalamus, and reduces the release of luteinizing hormone (LH) by the anterior pituitary gland. At this stage, levels of cGnIH receptor (cGnIHR) and cGnRH receptor III (cGnRHR-III) in the pituitary gland are highest and lowest, respectively. Due to the lack of stimulation, the reproductive tract (including the gonads) remains immature; B = upon photostimulation with long days, a reduction in MEL production by the retina of the eye and the pineal gland reduces cGnIH release, thus lifting the inhibition. Simultaneously, the increase in photoperiod activates deep brain (hypothalamic) photoreceptors, which indirectly stimulate the synthesis and release of cGnRH-I. In turn, this switch from inhibitory to stimulatory control results in increased production of LH, triggering the maturation of the gonads and consequently the release of sex steroid hormones (estradiol E2 and progesterone P4). As the bird matures sexually, E2 and P4 inhibit the expression of the cGnIHR in the pituitary gland, while an increase in cGnRHR-III expression is observed. At this stage, the hypothalamus is primarily under stimulatory control and the anterior pituitary gland is sensitive primarily to cGnRH.
of the reproductive axis. As day length increases, the reduction in melatonin secretion decreases GnIH production, removing its inhibitory input. Simultaneously, hypothalamic photoreceptors, possibly more sensitive to light from the red spectrum, indirectly trigger the activation of cGnRHR-II neurons resulting in the activation of the HPG. Recruitment of the gonads and the associated steroid production feeds back on the pituitary gland to switch the ratio of receptors in favor of cGnRHR-III, thus further exacerbating the stimulatory pathway and completing sexual maturation. On a practical level, this implies that to maximize egg production, lighting systems should be designed to specifically activate the stimulatory branch. Light-emitting diode (LED) technology may be the perfect tool for such “spectrum specific lighting” and over the last 5 years, several commercial products have entered the market. However, although lighting and photoperiod is one of the primary cues involved in the control of HPG, it is clear that other internal signals such as body weight may be equally important, and a complex neuronal network is most likely responsible for GnRH and GnIH production. The full extent and content of this network still needs to be characterized, and may ultimately resemble the mammalian KNDy model.

REFERENCES


