«Review»

Gonadotropin Releasing Hormones and their Receptors in Avian Species

Grégoy Y. Bédécarrats¹, Mamiko Shimizu¹ and Daniel Guémené²

¹ Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, N1G2W1, Canada

² Unité de Recherches Avicoles, INRA, 37380 Nouzilly, France

In all vertebrates, gonadotropin releasing hormone (GnRH) is a key component of the hypothalamo-pituitary-gonadal axis. In birds, two distinct forms of GnRH (cGnRH-I and cGnRH-II) have been characterized, and a third immunoreactive form similar to lamprey GnRH-III has been reported. Each GnRH is produced by a different population of neurons. cGnRH-I is predominantly synthesized in cells located in the preoptic and septal areas, whereas cGnRH-II and -III are produced in neurons located mainly in the mesencephalon and around the third ventricle, respectively. Although GnRH neurons have been shown to project fibers to the median eminence, it is still unclear whether all three peptides are released in the hypothalamo-pituitary portal vascular system. Nonetheless, *in vitro* as well as *in vivo* experiments confirmed that they all have the ability to induce the release of gonadotropins. In the anterior pituitary gland, GnRHs mediate their effects by binding to specific G-coupled protein receptors, and in chickens, two different receptors have been characterized (cGnRHR-I and cGnRHR-II). In addition to the full-length receptors, several splice variants have also been detected. Although the physiological relevance of these variants is not known, they may participate in the regulation of intracellular signaling. While the expression of the full-length cGnRHR-I appears to fluctuate during an ovulatory cycle, levels remain constant between reproductive stages in chickens. In contrast, pituitary levels of cGnRHR-II mRNA are directly correlated with the reproductive status of the bird. Whether these two GnRHRs are expressed by the same cells in the anterior pituitary gland is not known. However, since luteinizing hormone (LH) and follicle stimulating hormone (FSH) are produced by two different cell populations in chickens, it is possible that different combinations of GnRHs and GnRHRs differentially mediate the synthesis and release of these gonadotropins.

Key words : GnRH, GnRH receptor, reproduction

J. Poult. Sci., 43 : 199–214, 2006

Introduction

As for most vertebrates, reproduction in avian species is controlled by the hypothalamo-pituitary-gonadal axis. The physical and physiological events associated with the reproductive cycle of birds have been reviewed and described in detail elsewhere (Etches, 1996 ; Chaiseha and El Halawani, 2005). Briefly, upon photostimulation, gonadotropin releas-

ing hormone (GnRH) is released by the hypothalamus and stimulates the synthesis and release of gonadotropins (luteinizing hormone LH and follicle stimulating hormone FSH) by the anterior pituitary gonadotropes. In turn, LH and FSH stimulate the development of the gonads, and activation of this axis ultimately results in the initiation and maintenance of egg/sperm production. In addition to GnRH, the recent discovery of a gonadotropin in-

Received : January 19, 2006, Accepted : February 24, 2006

Correspondence to : Dr. Grégoy Y. Bédécarrats, Department of Animal and Poultry Science, University of Guelph, Ontario, N1G 2W1, Canada

Tel : 519-824-4120 ext. 53692 Fax : 519-837-8867 E-mail : gbedecar@uoguelph.ca

hibiting hormone (GnIH) has allowed the elaboration of new theories on the hypothalamic control of gonadotropins (Tsutsui *et al.*, 2000). The implication of GnIH in birds' reproduction is the subject of a separate review published in this issue of the Journal of Poultry Science.

In both vertebrates and invertebrates, multiple forms of GnRH have been isolated, and with the exception of the Octopus vulgaris (Iwakoshi *et al.*, 2002), they all correspond to decapeptides with a relatively well conserved sequence (Somoza *et al.*, 2002). Based on brain localization as well as species distribution, GnRH peptides can be classified into three major categories; a hypothalamic specific form (GnRH-I) present mainly in the preoptic area, an "ancient" form conserved throughout species (GnRH-II) present mainly in the mesencephalon, and a GnRH-III form present in the telencephalon of some species (Somoza *et al.*, 2002; Millar *et al.*, 2004). As for GnRH, multiple forms of its receptor (GnRHR) exist, and although most types of GnRH are able to bind and activate every GnRHR, differences in affinity and specificity are observed (Barran *et al.*, 2005). Among vertebrates, GnRHRs can be subdivided into mammalian and non-mammalian receptors. The most striking difference between the two being the lack of an intracellular C-terminal tail in the mammalian forms. This is of importance since the C-terminal tail was shown to be responsible for faster internalization (Pawson *et al.*, 1998). In addition, several specific micro-domains have also been shown to harbor ligand specificity (Wang *et al.*, 2004).

Since reproductive efficiency is central to a successful poultry industry, GnRH and its effects on the reproductive axis have been extensively studied in avian species and were previously reviewed by Dunn and Millam (1998) and Sharp *et al.* (1998). However, compared to mammals, few studies have focused on the structure/function of the GnRHRs in birds. This review summarizes earlier work on the identification and distribution of avian GnRHs, the effects of GnRHs on gonadotropins, and the characterization and function of GnRHRs.

Identification of GnRH Variants in Avian Species

In birds, three major types of GnRH have been isolated. However, whether all three forms directly

regulate pituitary gonadotropes remains controversial.

Evidence supporting the existence of GnRH in chickens was originally obtained from immunological studies (King and Millar, 1979), and cGnRH-I was first purified from chickens' hypothalami (Miyamoto *et al.*, 1982). Its structure and amino acid sequence were further determined (King and Millar, 1982; Miyamoto *et al.*, 1983), and it was shown to differ from the mammalian form by a substitution of an Arg with a Gln at position 8. As its mammalian counterpart, cGnRH-I is encoded by a gene composed of four 4 exons, leading to the synthesis of an 89 amino acid prepropeptide (Dunn *et al.*, 1993). In addition, a splice variant with retention of intron I which could result in a normally translated product was also identified (Sun *et al.*, 2001a). Processing of cGnRH-I prepropeptide and the possible role of its cryptic associated peptide have been previously reviewed (Dunn and Millam, 1998), including the specific enzymatic cleavage of the signal peptide, and the pyrolysis and amidation of the N- and C-terminal residues, respectively. The function of the associated cryptic peptide still remains to be determined, and although it may allow for normal processing of cGnRH-I, an endocrine role cannot be excluded.

In 1984, Miyamoto *et al.* identified a second GnRH (cGnRH-II) from chicken hypothalamic extracts. This cGnRH-II differs from cGnRH-I at position 5, 7 and 8. Interestingly, this form appears to be fairly conserved among vertebrates, and has been detected in several extra-hypothalamic tissues. Thus, it is thought to be the most ancient form of GnRH with multiple possible functions (Millar *et al.*, 2004). However, although GnRH-II sequence is ubiquitous, cloning of the chicken gene revealed that the prepropeptide differs greatly between mammals and avian species (Ikemoto and Park, 2005). In addition, as the cGnRH-II gene partly overlaps with another gene (MRPS26), five different transcripts have been identified, with three of them possibly generating a mature decapeptide (Ikemoto and Park, 2005). Based on immunocytochemistry studies, a third form of GnRH (similar to lamprey GnRH-III) has also been detected in the brain of songbirds (Bentley *et al.*, 2004). Although the biochemical structure of this new form of GnRH has not been characterized, it appears to bind specifi-

cally to antibodies raised against lamprey GnRH-III. Furthermore, preabsorption of the lamprey GnRH-III antiserum with cGnRH-I and II did not reduce binding to immunoreactive cells (Bentley *et al.*, 2004).

Distribution of GnRH Variants

Brain distribution, presence in the median eminence, and release in the hypothalamo-pituitary portal vascular system are all critical factors that dictate physiological relevance of GnRHs. Unfortunately, most localization studies rely on the use of antibodies with various degrees of specificity and sensitivity, and the distribution of cGnRHs still remains controversial.

The ontogeny of GnRH-I neurons in birds have previously been extensively reviewed by Norgren (1996) and Dunn and Millam (1998). In the chicken, as reported in many other species, GnRH-I neurons originate from the olfactory placode where they can be detected as early as embryonic day 3 to 5 (Murakami *et al.*, 1991 ; Norgren *et al.*, 1992 ; Sullivan and Silverman, 1993). These early GnRH-I cells then migrate into the brain area along the olfactory nerve, and reach the septum by embryonic day 7. From then on, cell bodies reach their adult location in the brain, and start projecting their axons toward the median eminence. Interestingly, the concentration of cGnRH-I in the chick brain decreases between embryonic day 8 and 12, and then starts increasing after day 17 (Millam *et al.*, 1993 a). It is possible that this reduction in immunoreactive GnRH-I results from gonadal steroid negative feedback during the maturation of the hypothalamo-pituitary-gonadal axis. In contrast to cGnRH-I, cGnRH-II levels remain extremely low in the brain of chicks until embryonic day 14, drastically increasing up until hatch (Millam *et al.*, 1993 a). To date, no evidence suggests that cGnRH-II neurons also originate in the olfactory placode. However, cGnRH-II antisera used in immunohistochemical studies may lack the sensitivity to detect extremely low amounts of this peptide. Sensitivity of antiserum is a critical issue and is most likely responsible for the lack of data regarding the presence of cGnRH before the formation of the olfactory placode. However, using more sensitive techniques such as RT-PCR and *in situ* hybridization, Witkin *et al.* (2003) detected the expression of cGnRH-I gene

in chicken embryos along the primitive streak before gastrulation, followed by the anterior neural ridge and the olfactory placode. These observations strongly suggest that cGnRH-I precursors may be committed as early as HH stage 4.

In adults, although early immunohistological studies using antibodies raised against mGnRH confirm the presence of cGnRH perikarya in the hypothalamus and other brain areas, as well as the presence of cGnRH fibers in the median eminence of several avian species, specific distribution of cGnRH-I and cGnRH-II neurons has not been determined (Dunn and Millam, 1998). Following the elucidation of the biochemical structure of both cGnRHs, specific antibodies against cGnRH-I and cGnRH-II were made available, and in 1988 Mikami *et al.* were the first to report a different distribution of immuno-reactive cGnRH-I and II cells in the quail and chicken brain. The two peptides appear to be present in two different populations of neurons with cGnRH-I perikarya located mainly in the preoptic and septal areas of both quail and chickens (Mikami *et al.*, 1988 ; Van Gils *et al.*, 1993), and the periventricular nucleus of chickens (Mikami *et al.*, 1988). In contrast, cGnRH-II perikarya were observed mainly in the rostral end of the mesencephalon around the oculomotor complex (Mikami *et al.*, 1988 ; Van Gils *et al.*, 1993). Similar results were also reported in the turkey (Millam *et al.*, 1993 b), where cGnRH-II neurons are located around the oculomotor complex extending to the mesencephalon. Kuenzel and Blahser (1991) further established the specific location of six major groups of cGnRH neurons throughout the brain. These include the olfactory bulb, olfactory tubercle, nucleus accumbens, three nuclei in the septal/preoptic hypothalamus (lateral septal and lateral ventricle areas, pallial commissure, preoptic/paraventricular nucleus), lateral anterior thalamic nucleus, and oculomotor complex (midbrain). However, these authors used an antibody raised against mammalian GnRH, and were thus not able to discriminate between cGnRH-I and cGnRH-II neurons.

To be physiologically relevant at the level of the pituitary, a given cGnRH needs to be present in the median eminence of the hypothalamus. Immunohistochemical evidence clearly demonstrates that cGnRH-I fibers project to the median eminence, especially cells from the pallial commissure. On the

other hand, whether axons from cGnRH-II neurons reach the median eminence is controversial, and although Mikami *et al.* (1988) and Millam *et al.* (1993a) failed to detect any cGnRH-II immunoreactive fibers in the median eminence of quail and chickens, Van Gils *et al.* (1993) and D'Hondt *et al.* (2001) were able to. Radioimmunoassay measurement of cGnRHs in chicken brain confirmed immunohistochemical evidence that cGnRH-I is significantly more abundant than cGnRH-II in the hypothalamus (Katz *et al.*, 1990; Sharp *et al.*, 1990), and that cGnRH-II is not (Sharp *et al.*, 1990) or barely (Katz *et al.*, 1990) present in the median eminence. Similarly, in Japanese quail, cGnRH-I concentrations in individual median eminences are far greater than that of cGnRH-II (Millam *et al.*, 1998). However, using the same antibody as Van Gils *et al.* (1993) and D'Hondt *et al.* (2001), Clerens *et al.* (2003) detected and purified cGnRH-II from the median eminence of quail. In addition to cGnRH-I and II, a new immunoreactive cGnRH-III similar to lamprey GnRH-III has also been detected in the brain of songbirds (Bentley *et al.*, 2004), and cGnRH-III neurons appear to be present in multiple areas with a higher density around the third ventricle. Interestingly, axons were also shown to project to the median eminence. It is worth mentioning that lamprey GnRH-III immunoreactive neurons were distinct from cGnRH-I and II cells (Bentley *et al.*, 2004).

In order to exert their effects on gonadotropins, cGnRHs need to reach the pituitary gland, and thus be released in the hypothalamo-pituitary portal blood system. Perfusion of hypothalamic slices containing the median eminence were shown to release GnRH in quail (Millam *et al.*, 1984), and both cGnRH-I and II were shown to be present in the posterior pituitary of turkeys (Rozenboim *et al.*, 1993). However, as reported by Millam *et al.* (1998), the release of cGnRHs from hypothalamic explants in perfusion may not necessarily represent the median eminence release, but may rather represent leakage from other hypothalamic structures. Interestingly, as reported in several mammalian species, mRNAs for cGnRH-I (Sun *et al.*, 2001) and cGnRH-II (Ikemoto and Park, 2005) were also detected in the pituitary gland of chickens. Although these levels appear to be minimal, this raises

the possibility that GnRHs may act as paracrine/autocrine factors within the pituitary.

Control of GnRHs Synthesis and Release

GnRHs are mainly associated with the development and function of the reproductive axis in avian species. Thus, the control of their synthesis and release will be discussed in the context of a reproductive cycle. Although birds can sexually mature spontaneously, the hypothalamo-pituitary-gonadal axis can be prematurely stimulated by increasing the photoperiod (photostimulation), with a transfer from short (7 h) to long (14 h) photoperiod providing maximum stimulation in chickens (Lewis *et al.*, 2002). During the onset of puberty or acquisition of sexual maturity, the abundance of cGnRH-I mRNA and peptide increase in the hypothalamus of chickens (Sharp *et al.*, 1990; Dunn and Sharp, 1999) and the basal hypothalamus of starlings (Dawson *et al.*, 2002). An increase in GnRH release can also be observed in superfused hypothalamic slices from photostimulated quail (Millam *et al.*, 1984), and this increase occurs within 23 h post photo-stimulation in hypothalamic explants of quail (Perera and Follett, 1992). At a histological level, the number of cGnRH-I fibers were shown to increase in the hypothalamus of breeding house sparrows when compared to non-breeding and juvenile birds (Stevenson and Macdougall-Shackleton, 2005). Similarly, in the same study, cGnRH-II fibers were also more abundant in the pre-optic area of breeding house sparrows, suggesting a plastic reorganization of neurons during sexual maturation. However, a role for cGnRH-II during sexual maturation still remains controversial, and Sharp *et al.* (1990) did not observe any changes in the hypothalamic content of peptide during the onset of puberty in cockerels. Nonetheless, despite the controversy surrounding cGnRH-II, it is clear that exposure to light controls the release of GnRH via hypothalamic photoreceptors (Sharp, 2005). Unfortunately, these receptors have been elusive and still remain to be identified. As suggested by Wilson (1997), thyroid hormones may also play an important role in the control of photoperiodism and the associated changes in GnRH-I synthesis and release during a reproductive cycle in sparrows. This was confirmed by the fact that thyroidectomy abolishes the increase in GnRH-I observed after photostimulation (Daw-

son *et al.*, 2002). Interestingly, the mechanisms by which thyroid hormones control GnRH neurons seem to reside within the hypothalamus, and photostimulation stimulates the conversion of T4 to T3 in the mediobasal area in quail (Yoshimura *et al.*, 2003). In turn, this increase in T3 induces morphological changes in the median eminence, allowing GnRH neurons to contact the peri-capillary space (Yoshimura, 2004). These findings have allowed the elaboration of new theories on how light stimulates the release of GnRH, thus stimulating the hypothalamo-pituitary-gonadal axis (for review : Yoshimura, 2004 ; Sharp, 2005). In adult hens, sexual maturity is characterized by the beginning of egg-laying. Ovulation in avian species is induced by an LH surge caused by positive feed-back from progesterone released by the dominant follicle (Wilson and Sharp, 1976). Whether GnRH plays an important role in mediating the progesterone effect on LH surges remains uncertain. In chickens and turkeys, laying rate progressively decreases with age, and hens will either enter a photorefractory period or express incubation behavior. No decrease in hypothalamic cGnRH-I mRNA or peptide content occur during the progressive cessation of egg-laying in chickens (Sharp *et al.*, 1992 ; Ciccone *et al.*, 2005). However, a significant decrease in mRNA levels was observed between laying and incubating hens (Dunn *et al.*, 1996), although this was not associated with changes in peptide content. In the turkey, a decrease in cGnRH-I and II peptide content in the hypothalamus was observed between laying or incubating hens and photorefractory hens (Rozenboim *et al.*, 1993). These observations are somewhat contradictory to the reports of Millam *et al.* (1989) and Millam *et al.* (1993b), who showed that the hypothalamic content in cGnRH-I and II is the highest during incubation in the turkey. These discrepancies may be explained by the fact that different hypothalamic regions were investigated, and whether the median eminence was included. In addition, mRNA levels do not necessarily correlate with cellular peptide content and are often not representative of the amount of peptide released. In the quail, cGnRH-I concentrations decrease in the median eminence of aging birds, and although concentrations in the preoptic area remain similar, cGnRH-I release is reduced in older birds (Ottinger *et al.*, 2004). Thus, it seems likely that different

mechanisms control gene expression, storage in the median eminence, and release of cGnRHs. A model for the effects of genotype dependent inputs from a biological clock on the secretion of GnRH in long and short day breeders has recently been proposed (Sharp, 2005). In this model, both stimulatory and inhibitory inputs affect the release of GnRH, and the impact of these factors is under the control of an internal biological clock as well as under the influence of external stimuli such as photoperiod. However, in addition to the positive and negative inputs from the biological clock, ovarian steroids may play a major control role once active egg-laying is initiated (Figure 1).

Effects of GnRHs on Gonadotropins

Although it remains unclear whether all three GnRHs are released into the hypothalamo-pituitary portal blood system, several studies confirmed that they all have the ability to stimulate pituitary gonadotropes. In quail and chickens, both cGnRH-I and II stimulate the release of LH and FSH *in vitro* as well as *in vivo* (Hattori *et al.*, 1986), and in sparrows a homolog to lamprey GnRH-III can also stimulate the release of LH *in vivo* (Bentley *et al.*, 2004). However, their relative potency is debatable, and cGnRH-I was shown to be as potent as cGnRH-II in stimulating LH release *in vitro* from immature cockerel (Hattori *et al.*, 1986) and in out-of-lay turkey hens (Guémené and Williams, 1999) pituitaries. On the other hand, cGnRH-II appears to be the most potent LH releasing hormone on cultured dispersed chicken pituitary cells (Chou *et al.*, 1985 ; Millar *et al.*, 1986) and perfused pituitaries from males (Guémené and Williams, 1992a) and incubating turkey hens (Guémené and Williams, 1999). Similarly *in vivo*, cGnRH-II has been shown to be more potent than cGnRH-I in both chickens (Wilson *et al.*, 1989 ; Proudman *et al.*, 2005) and male turkeys (Guémené and Williams, 1992a). However, no difference in potency was observed after injection of cGnRH-I and II in cockerels (Chou *et al.*, 1985), while cGnRH-II was shown to be more potent in male turkeys (Guémené and Williams, 1992a). On the other hand, cGnRH-I was as or more potent than cGnRH-II in the minutes following injection in turkey hens, while cGnRH-II was longer lasting (Guémené and Williams, 1999). Although results from these studies

Fig. 1. **A model for the interaction of cGnRHs with cGnRHRs, and the resulting effects on gonadotropin release.** The relative input of each component is represented by the thickness of lines and arrows. Dotted lines correspond to putative inputs. A, corresponds to an immature reproductive state. At this stage, GnRH-I-II and -III synthesis and release by the hypothalamus is minimal, and all three peptides have potentially the ability to bind to GnRHR-I and -II to maintain low levels of gonadotropins. In turn, low levels of LH and FSH result in minimal hormone production from the gonads which maintain a positive/negative feed-back on the hypothalamus and the pituitary gland. B, during the acquisition of sexual maturity following photostimulation, levels of cGnRHs secretion by the hypothalamus increase, and activation of the cGnRHR-I results in an increase in LH release by the pituitary. Simultaneously, levels of cGnRHR-II increase in the pituitary followed by an increase in FSH release. Both gonadotropins then induce the maturation of the gonads which ultimately results in spermatogenesis and ovulation. Once mature, gonads secrete steroid hormones which further regulate the secretion of LH by both negative and positive feed-back, whereas gonadal hormones inhibit FSH release. C, as the active reproductive phase progresses, cGnRHs secretion by the hypothalamus decreases reducing the stimulation of LH through cGnRHR-I. In addition, levels of cGnRHR-II in the pituitary decrease resulting in a lack of FSH stimulation. At this stage, gonadal steroids' positive and negative feed-backs on LH release are sufficient to maintain successive ovulation and egg laying.

appear to be contradictory, it is worth mentioning that species, sex, and reproductive status need to be taken into consideration. For example, intravenous injection of cGnRH-II is more effective in triggering the release of LH in hens than in cockerels (Sharp *et al.*, 1987), while the opposite is true in turkeys (Guémené and Williams, 1992 ab, 1999). Furthermore, perfused pituitaries from incubating turkey hens are more responsive to cGnRH-II than cGnRH-I whereas no difference in potency is observed with pituitaries from out-of-lay hens, and

pituitaries from laying hens failed to respond to cGnRH (Guémené and Williams, 1999). Interestingly in an earlier study, pituitaries from laying turkey hens were shown to respond to mammalian GnRH (Guémené and Williams, 1992 b).

Although cGnRHs are clearly able to stimulate the release of LH, their role in the control of FSH is more questionable. A positive effect of GnRH on FSH release was reported in dispersed chicken pituitary cells (Millar *et al.*, 1986) as well as *in vivo* and *in vitro* in quail (Hattori *et al.*, 1986). However,

little or no significant increase in circulating levels of FSH could be induced by injecting either cGnRH-I, -II or lamprey GnRH-III in chickens (Krishnan *et al.*, 1993 ; Dunn *et al.*, 2003 ; Proudman *et al.*, 2005). The discrepancy observed between these studies may result from the various degrees of specificity of radioimmunoassays used for the measurement of chicken FSH, as the assay used by Hattori *et al.* (1986) was shown to partly cross-react with LH (Sakai and Ishii, 1983). However, as mentioned for the control of LH secretion, sex, age and reproductive status may also be a determining factor. Most injections of GnRH that failed to induce the release of FSH were performed on non-photostimulated pullets (Dunn *et al.*, 2003), pre-pubertal pullets (Proudman *et al.*, 2005), and non-laying hens or immature cockerels (Krishnan *et al.*, 1993). Interestingly, an increase in circulating levels of FSH was observed when high doses of cGnRH-II were injected in 32 weeks old mature roosters (Proudman *et al.*, 2005). As described in the following chapter, large variations in GnRHR-II mRNA levels are observed in the pituitary gland of chickens during a reproductive cycle (Shimizu and Bédécarrats, 2005), and it is thus possible that varying amounts of GnRHRs differentially mediate the effect of cGnRHs on LH and FSH secretion. This is an attractive hypothesis (presented in Figure 1), as LH and FSH have been shown to be produced by two different populations of gonadotropes (Proudman *et al.*, 1999 ; Puebla-Osorio *et al.*, 2002). In ganders, intramuscular injection of Phe6-GnRH-Ethylamide (a GnRH agonist) between photostimulation and sexual maturity did not significantly affect circulating levels of LH (Sellier *et al.*, 1995). However, it did transiently decrease levels of testosterone, increase the fertility during the second half of a reproductive period, and partially prevented the natural decline in sperm production (Sellier *et al.*, 1995). Although FSH levels were not measured, it appears that the GnRH effects observed in the latter study were independent of the release of LH.

Nonetheless, the most convincing evidence regarding the relative physiological relevance of cGnRHs in chickens is probably the gonadal reduction observed after the active immunization of laying hens against cGnRH-I and not cGnRH-II, pointing at cGnRH-I being “the” gonadotropin releasing factor (Sharp *et al.*, 1990).

In birds as in mammals, GnRH secretion is pulsatile, and *in vitro* perfused hypothalamic sections release GnRH-I at a frequency of approximately 3 pulses per hour (Li *et al.*, 1994). Furthermore, the pulsatility center appears to be located in the medio-basal hypothalamus and the preoptic area (Li *et al.*, 1994). Pulsatility is also observed *in vivo* where GnRH pulse frequency can be deduced from profiles of circulating LH. In quail, LH pulses could be observed only after exposure to long days with a frequency of 1 pulse per 80 min (Urbanski, 1984). In turkey hens, pulsatile release of LH is observed before photostimulation with an average of 4 to 6 pulses per 23 hours. However, following photostimulation, basal levels significantly increase and pulses become less apparent (Chapman *et al.*, 1994). A lack of detectable discrete LH pulses in laying turkey hens has also been reported (Yang *et al.*, 1997). In broiler breeders, pulsatile LH release could be observed at an average frequency of 2 pulses per hour in males (Vizcarra *et al.*, 2004) and 1 pulse per hour in females at the end of a laying period (Senthilkumaran *et al.*, 2005). Interestingly, in male broiler breeders, the profile of FSH pulses appears to be independent from that of LH, again suggesting a different regulation between the two gonadotropins (Vizcarra *et al.*, 2004). Taken together, these studies suggest that, conversely to mammals, in which varying GnRH pulse frequency differentially regulates LH and FSH synthesis and release during an ovarian cycle (Levine and Ramirez, 1982 ; Haisenleder *et al.*, 1991 ; Bédécarrats and Kaiser, 2003), the initiation and maintenance of egg-laying in avian species is mainly associated with an increase in LH baseline concentrations rather than an increase in pulse frequency. Nonetheless, continuous GnRH exposure or stimulation at a frequency exceeding 1 pulse/30 min induces the desensitization of diced pituitary glands in perfusion (Guémené and Williams, 1992 ab ; Guémené and Williams, 1999), suggesting that as observed in mammals (Belchetz *et al.*, 1978), pulsatile GnRH is required to effectively stimulate pituitary gonadotropes.

Isolation of GnRH Receptors and their Splice Variants in Avian Species

The fact that cGnRHs are able to specifically induce the release of LH and FSH *in vivo* as well as

in vitro, and that pituitary gonadotropes can be desensitized by continuous stimulation suggests that cGnRHs act through specific cell surface receptors. Evidence of specific binding of cGnRH-I to chicken pituitary cell membranes was first reported by Millar and King (1983). Interestingly, the affinity of a rat pituitary cell membrane preparation for cGnRH-I appeared to be lower than for its mammalian counterpart (Millar and King, 1983). Binding characteristics of cGnRH-I to a chicken pituitary cell membrane preparation were characterized by Kawashima *et al.* (1992). The dissociation constant (K_d) as well as the maximum binding (B_{max}) appeared to fluctuate with the reproductive stage as well as the ovulatory status of the hen, suggesting changes in the number of cell surface binding sites and the existence of possibly more than one receptor. The first cGnRHR (designated as cGnRHR-I in this review) to be cloned was originally isolated from adult cockerels (Sun *et al.*, 2001 a). As in other vertebrates, this receptor belongs to the G-protein-coupled receptor (GPCR) family, with its characteristic seven transmembrane domains. However, the existence of at least two GnRHs within a species suggests that cognate receptors are present. As a matter of fact, many vertebrates from amphibians and fish (Illing *et al.*, 1999 ; Troskie *et al.*, 2000 ; Jodo *et al.*, 2005) to mammals (Millar *et al.*, 2001 ; Neill *et al.*, 2001 ; Morgan *et al.*, 2003) possess more than one receptor. Thus, it is likely that additional GnRHRs also exist in avian species, an assumption that has been supported by the assembled chicken genome. We have recently cloned a novel cGnRHR (designated as cGnRH-II, Figure 2, Genbank accession number : AY895154) from the pituitary gland of a mature White Leghorn male (Shimizu and Bédécarrats, 2005). Unlike mammalian GnRHR genes which consist of 3 exons and 2 introns (Zhou and Sealfon, 1994 ; Kakar, 1997), both cGnRHR-I and II genes are composed of 4 exons and 3 introns encoding a 375 and 419 amino acid protein, respectively. At the amino acid level, cGnRHR-II shares 53% homology with the cGnRHR-I and 51–65% homology with the amphibian and fish receptors. As for other non-mammalian receptors, cGnRHRs possess a carboxy-terminal tail which has been shown to be responsible for desensitization and rapid internalization (Pawson *et al.*, 1998 ; Blumenrohr *et al.*, 1999 ; Pawson *et al.*,

2003).

In addition to the full length receptors, multiple splice variants (SVs) of GnRHRs have been identified in many vertebrate species. These variants correspond to either shorter or longer transcripts. For example, one SV from bullfrog (bf) GnRHR-III is generated by exon skipping, whereas two others arise from intron retention and a fourth from transcriptional slippage (Wang *et al.*, 2001). Alternative splicing was also reported in the mouse (Zhou and Sealfon, 1994) and human GnRHRs (Kottler *et al.*, 1999) in which complete deletion of exon 2 was observed. Similarly in chickens, multiple SVs have been reported, and a SV of cGnRHR-I resulting in the deletion of 90% of exon II (Sun *et al.*, 2001 a), and two SVs of cGnRHR-II (Figure 2) corresponding to the deletion of exons 2/3 and part of exon 2 were identified (Shimizu and Bédécarrats, 2005).

Splice variants of the bfGnRHRs were shown to be present in both the cell membrane and cytoplasm of transfected HeLa cells, and to inhibit signalling of the full length receptor, suggesting physical interactions between receptors (Wang *et al.*, 2001). Similarly, a truncated human GnRHR-II consisting of part of transmembrane domain 5 to the carboxy terminus was shown to inhibit signaling of the full length receptor in a dose-dependent manner when coexpressed in COS-7 cells (Pawson *et al.*, 2005). The mechanisms by which these variants inhibit signalling of the full length receptors are not known. However, as suggested by Pawson *et al.* (2005), it is possible that these SVs alter the normal *de novo* synthesis of the full length receptors, possibly at the level of the nucleus, endoplasmic reticulum, or Golgi. It thus appears that an important relationship exists between subcellular localization of SVs and their inhibitory effect. Using fluorescence resonance energy transfer, Cheung and Hearn (2005) showed that both full length and SVs of wallaby GnRHR form homo- as well as heterodimers. Furthermore, microaggregation between GnRHRs is promoted by GnRH agonist as a part of normal receptor signal transduction (Cornea *et al.*, 2001). Although the functional relevance of SVs needs to be elucidated further, these studies support the hypothesis that SVs may be important in regulating full-length receptor signal transduction.

Fig. 2. Schematic representation of the full length cGnRHR-II (panel A) and its splice variants (SV1 and SV2, panels B and C, respectively). Rectangular boxes represent cell plasma membrane. Each circle corresponds to an individual amino acid. Shaded amino acids correspond to sequences suspected of being important for receptor-ligand interaction (D and N in transmembrane domain 2), differential ligand selectivity (P-P-S in extracellular loop 3), and receptor activation (D-R-X-X-X-I/V-X-X-P-L in intracellular loop 2 ; D-P-X-X-Y in transmembrane domain 7). Single head arrows show cystein residues which may be involved in disulfide bond formation, whereas (*) show potential glycosylation sites. Double headed arrows correspond to the deletion site within splice variants.

Distribution of GnRHRs and Control of their Gene Expression

Most species with multiple GnRHRs possess at least one pituitary-specific form (Millar *et al.*, 2004). For example, in the bullfrog, three types of receptors were identified. bfGnRHR-I was shown to be pituitary-specific, whereas bfGnRHR-II and -III are expressed in the forebrain and hindbrain (Wang *et al.*, 2001). Thus, bfGnRHR-I may play a role in the neuroendocrine control of gonadotropin secretion, and bfGnRHR-II and -III may regulate other brain functions. In general, the “type-II GnRHRs” are widely distributed in the brain, as well as other tissues such as the pancreas, lung, and kidney (Millar, 2003). This is the case for cGnRHR-I, which is expressed in the brain, the pituitary, testes, spleen, and heart (Sun *et al.*, 2001 a). In contrast, expression of cGnRHR-II was detected in the pituitary gland but not in brain tissues from both males and females (Shimizu and Bédécarrats, 2005). The SV of cGnRHR-I was also shown to be expressed in the same tissue as the full length receptor. Interestingly, mRNA encoding the longest variant of cGnRHR-II was predominantly detected in the pituitary, whereas mRNA from the shortest variant was detected in most brain tissues examined (Shimizu and Bédécarrats, 2005).

As mentioned previously, *in vivo* and *in vitro* responsiveness to cGnRHs may depend on such factors as sex and age. Interestingly, primary culture of chicken gonadotropes collected from juvenile birds seem to better regain their responsiveness to GnRH-I than cells harvested from adults (Liu *et al.*, 1996). However, the changes in Bmax and Kd observed in pituitary cell membrane preparations derived from hens at different ovulatory stages (Kawashima *et al.*, 1992) suggest that gene expression of cGnRHRs and cell surface localization are regulated in chicken pituitaries. In fact, an increase in cGnRHR-I mRNA levels is observed in the pituitary of hens throughout a 20 hour period preceding ovulation, with maximum levels observed around the time of the LH surge (Lovell *et al.*, 2005). We did not observe any differences in cGnRHR-I mRNA pituitary content between stages during a reproductive cycle. However, we did observe dramatic changes in cGnRH-II mRNA levels (Shimizu and Bédécarrats, 2005). In females, levels of

mRNA increase from the immature stage to the peak of lay, then decreasing toward the end of the reproductive cycle (Shimizu and Bédécarrats, 2005). Similarly, in males, levels of mRNA increase from the immature stage up to 4 weeks post photostimulation, decreasing thereafter (unpublished data). It thus appears that cGnRHR-I and -II gene expression are regulated by two different mechanisms. Whether these two receptors are expressed in the same pituitary cells is not known. However, it is possible that cGnRHR-I is involved in the secretion of LH during ovulatory cycles, while cGnRHR-II plays a role in the development and maturation of the reproductive axis post-photostimulation (Figure 1).

Molecular mechanisms involved in the control of mammalian GnRHR gene expression in various tissues have been extensively studied and were previously reviewed by Hapgood *et al.* (2005). Unlike their mammalian counterparts, the gene promoters of cGnRHRs have not been characterized and the mechanisms responsible for their expression remain to be determined. Nonetheless, castration of juvenile cockerels results in a significant increase in cGnRHR-I mRNA levels in the preoptic area and the anterior pituitary, and this increase is partially inhibited after treatment with oestrogen (Sun *et al.*, 2001 a). Interestingly, no changes in cGnRH-I mRNA levels were observed in the mediobasal hypothalamus. Furthermore, treatment of intact adults with oestrogen did not affect levels of cGnRH-I (Sun *et al.*, 2001 a). In non-laying hens, a single injection of ovarian steroid hormones (progesterone, estradiol or dihydrotestosterone) increases both the Kd and Bmax of pituitary cell membrane preparations (Kawashima *et al.*, 1992). Taken together, these results suggest that gonadal steroids have the ability to feed-back at the hypothalamus and pituitary level to control cGnRHR gene expression. However, whether these steroids control both receptors the same way is questionable, and once again, the sex and reproductive status of the bird may be critical factors.

Activation of cGnRHRs and Initiation of Intracellular Signalling

As members of the GPCR family, GnRHRs have the ability to activate multiple intracellular second messenger cascades depending on the type of G-

proteins involved. Coupling to $G_{\alpha q}$ activates the phospholipase C pathway, resulting in the hydrolysis of phosphatidylinositol 4, 5-bisphosphate (PIP_2) to produce inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG). In turn, IP_3 induces the release of calcium from endoplasmic stores, and DAG activates protein kinase C. Coupling to $G_{\alpha s}$ or $G_{\alpha i}$ activates and inhibits the adenylyl cyclase pathway, respectively, resulting in changes in cAMP production. In addition, binding of GnRH to its receptor can also activate L-type voltage sensitive calcium channels as well as non-voltage sensitive calcium channels (for review : Stojilkovic *et al.*, 1994 ; Millar *et al.*, 2004).

As expected, depletion of calcium in culture media or the use of calcium channel blockers reduces GnRH-induced release of LH from chicken pituitary cells (Davidson *et al.*, 1988 ; Liu *et al.*, 1995), and artificial activation of the PLC pathway increases the release of LH (Johnson and Tilly, 1991). These observations, coupled with the fact that stimulation of chicken pituitary cells with GnRH results in increased intracellular IP_3 levels (Davidson *et al.*, 1988), suggest association of cGnRHRs with $G_{\alpha q}$. This was further confirmed after the cloning of both cGnRHR-I and -II (Sun *et al.*, 2001 ; Shimizu and Bédécarrats, 2005). However, whether cGnRHRs can also couple to other G-protein is not known. Interestingly, bfGnRHR-II couples more actively to $G_{\alpha s}$ and bfGnRHR-I and -III couple to both $G_{\alpha q/11}$ and $G_{\alpha s}$ with similar efficiency, whereas rat GnRHR preferentially couples to $G_{\alpha q/11}$, suggesting that differences in structure and/or in the amino acid sequence in intracellular loops may direct GnRHRs toward different signalling cascades (Oh *et al.*, 2003). It is worth mentioning that cGnRHR-II is 61, 51 and 65% homologous to bfGnRHR-I, -II and -III, respectively, whereas cGnRH-I has 51, 63 and 51% homology with the same receptors. It is thus possible that in chicken, as observed in the bullfrog, different GnRHRs possess different affinity for $G_{\alpha q/11}$ and $G_{\alpha s}$.

The relative affinity of cGnRH-I and -II for the two chicken receptor types still needs to be determined. However, a high dose of mammalian GnRH or cGnRH-II stimulates IP_3 production to a greater extent in cells transfected with cGnRHR-II than cells transfected with cGnRHR-I (unpublished data). Type II GnRHRs have been shown to be

generally more sensitive to GnRH-II than to mammalian GnRH (Wang *et al.*, 2004), and in bullfrogs, bfGnRHR-I has a similar sensitivity to cGnRH II and mammalian GnRH whereas bfGnRHR-II and bfGnRHR-III show highest sensitivity for cGnRH-II and sGnRH, respectively (Wang *et al.*, 2001 ; Seong *et al.*, 2003). Likewise, cGnRHR-I has a higher affinity for cGnRH-II than mammalian GnRH, whereas the opposite is true for the human GnRHR (Sun *et al.*, 2001 b). Interestingly, treatment of laying hens with buserelin (a mammalian GnRH super-agonist) does not substantially enhance the increase in LH observed with cGnRH-I or mammalian GnRH injection (Sterling and Sharp, 1984). In addition, some mammalian GnRHR antagonists behave as agonists to stimulate LH release from cultured chicken pituitary cells (Jacobs *et al.*, 1995), and some human GnRHR antagonists act as agonists on cGnRHR-I in transfected COS-1 cells (Sun *et al.*, 2001 b). These experiments suggest that mammalian and chicken GnRHs and GnRHRs possess structural requirements responsible for specificity. Sun *et al.* (2001 b) suggested that D-Lys⁶ in mammalian antagonists interacts with the extracellular domain 2 of cGnRHR-I, and that this interaction is responsible for the agonist effect. Interaction between Arg⁸ of mammalian GnRH and an acidic residue in the extra-cellular loop 3 of mammalian receptors was proposed to be important for high affinity binding (Flanagan *et al.*, 1994). However, cGnRHR-I binds mammalian GnRH and [Gln⁸] GnRH with the same high affinity, suggesting that alternative sites are probably involved in the binding of cGnRHR-I to its ligands (Sun *et al.*, 2001 b).

Despite the difference in selectivity and affinity, several amino acid motifs important for ligand binding in other GnRHRs are also present in cGnRHRs (Figure 2). For example, Asp^{2.61(98)} and Asn^{2.65(102)}, which interact with the amino- and carboxy-terminal residues (pGlu-His, and Gly-NH₂, respectively) of GnRH (Davidson *et al.*, 1996 ; Flanagan *et al.*, 2000), are present in both cGnRHR-I and cGnRHR-II. A P-X-S/Y motif in extracellular loop 3 that was shown to be a determinant for differential ligand selectivity (Wang *et al.*, 2004) corresponds to a P-E-Y and P-P-S motif in cGnRHR-I and GnRHR-II, respectively. Furthermore, a D-RXXXI/VXXXP-L motif in intracellular loop 2, and a DPXXY motif in transmembrane domain 7 that

are important for receptor activation (Ballesteros *et al.*, 1998 ; Millar *et al.*, 2004) are also present in cGnRHRs. However, the Lys^{3,32 (121)} that was shown to be important for ligand binding in other receptors (Zhou *et al.*, 1995) is present only in cGnRHR-I.

References

- Ballesteros J, Kitanovic S, Guarnieri F, Davies P, Fromme BJ, Konvicka K, Chi L, Millar RP, Davidson JS, Weinstein H and Sealfon SC. Functional microdomains in G-protein-coupled receptors. The conserved arginine-cage motif in the gonadotropin-releasing hormone receptor. *Journal of Biological Chemistry*, 273 : 10445–10453. 1998.
- Barran PE, Roeske RW, Pawson AJ, Sellar R, Bowers MT, Morgan K, Lu ZL, Tsuda M, Kusakabe T and Millar RP. Evolution of constrained gonadotropin-releasing hormone ligand conformation and receptor selectivity. *Journal of Biological Chemistry*, 280 : 38569–38575. 2005.
- Bédécarrats GY and Kaiser UB. Differential Regulation of Gonadotropin Subunit Gene Promoter Activity by Pulsatile Gonadotropin-Releasing Hormone (GnRH) in Perfused LbetaT2 Cells : Role of GnRH Receptor Concentration. *Endocrinology*, 144 : 1802–1811. 2003.
- Belchetz PE, Plant TM, Nakai Y, Keogh EJ and Knobil E. Hypophysial responses to continuous and intermittent delivery of hypothalamic gonadotropin-releasing hormone. *Science*, 202 : 631–633. 1978.
- Bentley GE, Moore IT, Sower SA and Wingfield JC. Evidence for a novel gonadotropin-releasing hormone in hypothalamic and forebrain areas in songbirds. *Brain, Behavior and Evolution*, 63 : 34–46. 2004.
- Blomenrohr M, Heding A, Sellar R, Leurs R, Bogerd J, Eidne KA and Willars GB. Pivotal role for the cytoplasmic carboxyl-terminal tail of a nonmammalian gonadotropin-releasing hormone receptor in cell surface expression, ligand binding, and receptor phosphorylation and internalization. *Molecular Pharmacology*, 56 : 1229–1237. 1999.
- Chaiseha Y and El Halawani ME. Neuroendocrinology of the female turkey reproductive cycle. *Journal of Poultry Science*, 42 : 87–100. 2005.
- Chapman DP, Bacon WL, Long DW, Kurima K and Burke WH. Photostimulation changes the pattern of luteinizing hormone secretion in turkey hens. *General and Comparative Endocrinology*, 96 : 63–74. 1994.
- Cheung TC and Hearn JP. Dimerizations of the wallaby gonadotropin-releasing hormone receptor and its splice variants. *General and Comparative Endocrinology*, 144 : 280–288. 2005.
- Chou HF, Johnson AL and Williams JB. Luteinizing hormone releasing activity of [Gln8]-LHRH and [His5, Trp7, Tyr8]-LHRH in the cockerel, in vivo and in vitro. *Life Sciences*, 37 : 2459–2465. 1985.
- Ciccone NA, Sharp PJ, Wilson PW and Dunn IC. Changes in reproductive neuroendocrine mRNAs with decreasing ovarian function in ageing hens. *General and Comparative Endocrinology*, 144 : 20–27. 2005.
- Clerens S, D'Hondt E, Berghman LR, Vandesande F and Arckens L. Identification of cGnRH-II in the median eminence of Japanese quail (*Coturnix coturnix japonica*). *General and Comparative Endocrinology*, 131 : 48–56. 2003.
- Cornea A, Janovick JA, Maya-Nunez G and Conn PM. Gonadotropin-releasing hormone receptor microaggregation. Rate monitored by fluorescence resonance energy transfer. *Journal of Biological Chemistry*, 276 : 2153–2158. 2001.
- Davidson JS, McArdle CA, Davies P, Elario R, Flanagan CA and Millar RP. Asn102 of the gonadotropin-releasing hormone receptor is a critical determinant of potency for agonists containing C-terminal glycineamide. *Journal of Biological Chemistry*, 271 : 15510–15514. 1996.
- Davidson JS, Wakefield IK, King JA, Mulligan GP and Millar RP. Dual pathways of calcium entry in spike and plateau phases of luteinizing hormone release from chicken pituitary cells : sequential activation of receptor-operated and voltage-sensitive calcium channels by gonadotropin-releasing hormone. *Molecular Endocrinology*, 2 : 382–390. 1988.
- Dawson A, Talbot RT, Dunn IC and Sharp PJ. Changes in basal hypothalamic chicken gonadotropin-releasing hormone-I and vasoactive intestinal polypeptide associated with a photo-induced cycle in gonadal maturation and prolactin secretion in intact and thyroidectomized starlings (*Sturnus vulgaris*). *Journal of Neuroendocrinology*, 14 : 533–539. 2002.
- D'Hondt E, Billen J, Berghman L, Vandesande F and Arckens L. Chicken luteinizing hormone-releasing hormone-I and II are located in distinct fiber terminals in the median eminence of the quail : a light and electron microscopic study. *Belgium Journal of Zoology*, 131 : 137–144. 2001.
- Dunn IC, Beattie KK, Maney D, Sang HM, Talbot RT, Wilson PW and Sharp PJ. Regulation of chicken gonadotropin-releasing hormone-I mRNA in incubating, nest-deprived and laying bantam hens. *Neuroendocrinology*, 63 : 504–513. 1996.
- Dunn IC, Chen Y, Hook C, Sharp PJ and Sang HM. Characterization of the chicken preprogonadotrophin-releasing hormone-I gene. *Journal of Molecular Endocrinology*, 11 : 19–29. 1993.
- Dunn IC, Lewis PD, Wilson PW and Sharp PJ. Acceleration of maturation of FSH and LH responses to photostimulation in prepubertal domestic hens by oestrogen. *Reproduction*, 126 : 217–225. 2003.
- Dunn IC and Millam JR. Gonadotropin releasing hormone : forms and functions in birds. *Poultry and Avian Biology Reviews*, 9 : 61–85. 1998.
- Dunn IC and Sharp PJ. Photo-induction of hypothalamic gonadotrophin releasing hormone-I mRNA in the domestic chicken : a role for oestrogen? *Journal of Neuroendocrinology*, 11 : 371–375. 1999.
- Etches RJ. *Reproduction in poultry*. pp. 318. CAB International. Wallingford Englnd. 1996.

- Flanagan CA, Becker, II, Davidson JS, Wakefield IK, Zhou W, Sealfon SC and Millar RP. Glutamate 301 of the mouse gonadotropin-releasing hormone receptor confers specificity for arginine 8 of mammalian gonadotropin-releasing hormone. *Journal of Biological Chemistry*, 269 : 22636–22641. 1994.
- Flanagan CA, Rodic V, Konvicka K, Yuen T, Chi L, Rivier JE, Millar RP, Weinstein H and Sealfon SC. Multiple interactions of the Asp(2.61(98)) side chain of the gonadotropin-releasing hormone receptor contribute differentially to ligand interaction. *Biochemistry*, 39 : 8133–8141. 2000.
- Guémené D and Williams JB. In-vitro and in-vivo responses to chicken LHRH-I and chicken LHRH-II in male turkeys (*Meleagris gallopavo*). *Journal of Endocrinology*, 132 : 387–393. 1992 a.
- Guémené D and Williams JB. Comparison of the basal and luteinizing hormone-releasing hormone (LH-RH) induced luteinizing hormone (LH) release by perfused hypophyses from turkey hens (*Meleagris gallopavo*) at different physiological stages. *British Poultry Science*, 33 : 153–163. 1992 b.
- Guémené D and Williams JB. LH responses to chicken luteinizing hormone-releasing hormone I and II in laying, incubating, and out of lay turkey hens. *Domestic Animal Endocrinology*, 17 : 1–15. 1999.
- Haisenleder DJ, Dalkin AC, Ortolano GA, Marshall JC and Shupnik MA. A pulsatile gonadotropin-releasing hormone stimulus is required to increase transcription of the gonadotropin subunit genes : evidence for differential regulation of transcription by pulse frequency in vivo. *Endocrinology*, 128 : 509–517. 1991.
- Hapgood JP, Sadie H, van Biljon W and Ronacher K. Regulation of expression of mammalian gonadotrophin-releasing hormone receptor genes. *Journal of Neuroendocrinology*, 17 : 619–638. 2005.
- Hattori A, Ishii S and Wada M. Effects of two kinds of chicken luteinizing hormone-releasing hormone (LH-RH), mammalian LH-RH and its analogs on the release of LH and FSH in Japanese quail and chicken. *General and Comparative Endocrinology*, 64 : 446–455. 1986.
- Ikemoto T and Park MK. Molecular and evolutionary characterization of the GnRH-II gene in the chicken : Distinctive genomic organization, expression pattern, and precursor sequence. *Gene*, 2005.
- Illing N, Troskie BE, Nahorniak CS, Hapgood JP, Peter RE and Millar RP. Two gonadotropin-releasing hormone receptor subtypes with distinct ligand selectivity and differential distribution in brain and pituitary in the goldfish (*Carassius auratus*). *Proceedings of the National Academy of Sciences of the United States of America*, 96 : 2526–2531. 1999.
- Iwakoshi E, Takawa-Kuroda K, Fujisawa Y, Hisada M, Ukena K, Tsutsui K and Minakata H. Isolation and characterization of a GnRH-like peptide from *Octopus vulgaris*. *Biochemical and Biophysical Research Communications*, 291 : 1187–1193. 2002.
- Jacobs GF, Flanagan CA, Roeske RW and Millar RP. Agonist activity of mammalian gonadotropin-releasing antagonists in chicken gonadotropes reflects marked differences in vertebrate gonadotropin-releasing receptors. *Molecular and Cellular Endocrinology*, 108 : 107–113. 1995.
- Jodo A, Kitahashi T, Taniyama S, Ueda H, Urano A and Ando H. Seasonal changes in expression of genes encoding five types of gonadotropin-releasing hormone receptors and responses to GnRH analog in the pituitary of masu salmon. *General and Comparative Endocrinology*, 144 : 1–9. 2005.
- Johnson AL and Tilly JL. Second messenger pathways mediating chicken luteinizing hormone secretion from dispersed pituitary cells. *Biology of Reproduction*, 45 : 64–72. 1991.
- Kakar SS. Molecular structure of the human gonadotropin-releasing hormone receptor gene. *European Journal of Endocrinology*, 137 : 183–192. 1997.
- Katz IA, Millar RP and King JA. Differential regional distribution and release of two forms of gonadotropin-releasing hormone in the chicken brain. *Peptides*, 11 : 443–450. 1990.
- Kawashima M, Takeo H, Kamiyoshi M and Tanaka K. Luteinizing hormone-releasing hormone receptor bindings of the hen pituitary : difference between laying and nonlaying hens, effects of ovarian steroid hormones in vivo, and changes during an ovulatory cycle. *Poultry Science*, 71 : 1079–1086. 1992.
- King JA and Millar RP. Heterogeneity of vertebrate luteinizing hormone-releasing hormone. *Science*, 206 : 67–69. 1979.
- King JA and Millar RP. Structure of chicken hypothalamic luteinizing hormone-releasing hormone. II. Isolation and characterization. *Journal of Biological Chemistry*, 257 : 10729–10732. 1982.
- Kottler ML, Bergametti F, Carre MC, Morice S, Decoret E, Lagarde JP, Starzec A and Counis R. Tissue-specific pattern of variant transcripts of the human gonadotropin-releasing hormone receptor gene. *European Journal of Endocrinology*, 140 : 561–569. 1999.
- Krishnan KA, Proudman JA, Bolt DJ and Bahr JM. Development of an homologous radioimmunoassay for chicken follicle-stimulating hormone and measurement of plasma FSH during the ovulatory cycle. *Comparative Biochemistry and Physiology Part A Physiology*, 105 : 729–734. 1993.
- Kuenzel WJ and Blahser S. The distribution of gonadotropin-releasing hormone (GnRH) neurons and fibers throughout the chick brain (*Gallus domesticus*). *Cell and Tissue Research*, 264 : 481–495. 1991.
- Levine JE and Ramirez VD. LHRH release during the rat estrous cycle and after ovariectomy, as estimated with push-pull cannulae. *Endocrinology*, 111 : 1439–1448. 1982.
- Lewis PD, Morris TR and Perry GC. A model for predicting the age at sexual maturity for growing pullets of layer strains given a single change in photoperiod. *Journal of Agricultural Science*, 138 : 441–458. 2002.
- Li Q, Tamarkin L, Levantine P and Ottinger MA. Estradiol and androgen modulate chicken luteinizing hormone-

- releasing hormone-I release in vitro. *Biology of Reproduction*, 51 : 896-903. 1994.
- Liu RC, Lea RW and Sharp PJ. Sexually differentiated role of calcium ions in chicken GnRH-I-stimulated release of LH from anterior pituitary glands from adult domestic chickens. *General and Comparative Endocrinology*, 100 : 267-272. 1995.
- Liu RC, Sharp PJ and Lea RW. Effect of age of donor on the responsiveness of dispersed and cultured chicken anterior pituitary cells to GnRH-I. *British Poultry Science*, 37 : 451-463. 1996.
- Lovell TM, Knight PG and Gladwell RT. Variation in pituitary expression of mRNAs encoding the putative inhibin co-receptor (betaglycan) and type-I and type-II activin receptors during the chicken ovulatory cycle. *Journal of Endocrinology*, 186 : 447-455. 2005.
- Mikami S, Yamada S, Hasegawa Y and Miyamoto K. Localization of avian LHRH-immunoreactive neurons in the hypothalamus of the domestic fowl, *Gallus domesticus*, and the Japanese quail, *Coturnix coturnix*. *Cell and Tissue Research*, 251 : 51-58. 1988.
- Millam JR, Burke WH and El Halawani ME. Release of gonadotropin-releasing hormone from the Japanese quail hypothalamus in vitro. *General and Comparative Endocrinology*, 53 : 293-301. 1984.
- Millam JR, Craig-Veit CB, Adams TE and Adams BM. Avian gonadotropin-releasing hormones I and II in brain and other tissues in turkey hens. *Comparative Biochemistry and Physiology Part A Physiology*, 94 : 771-776. 1989.
- Millam JR, Craig-Veit CB and Petitte JN. Brain content of cGnRH I and II during embryonic development in chickens. *General and Comparative Endocrinology* 92 : 311-317. 1993 a.
- Millam JR, Faris PL, Youngren OM, el Halawani ME and Hartman BK. Immunohistochemical localization of chicken gonadotropin-releasing hormones I and II (cGnRH I and II) in turkey hen brain. *Journal of Comparative Neurology*, 333 : 68-82. 1993 b.
- Millam JR, Ottinger MA, Craig-Veit CB, Fan Y, Chaiseha Y and el Halawani M. Multiple forms of GnRH are released from perfused medial basal hypothalamic/preoptic area (MBH/POA) explants in birds. *General and Comparative Endocrinology*, 111 : 95-101. 1998.
- Millar R, Lowe S, Conklin D, Pawson A, Maudsley S, Troskie B, Ott T, Millar M, Lincoln G, Sellar R, Faurholm B, Scobie G, Kuestner R, Terasawa E and Katz A. A novel mammalian receptor for the evolutionarily conserved type II GnRH. *Proceedings of the National Academy of Sciences of the United States of America*, 98 : 9636-9641. 2001.
- Millar RP. GnRH II and type II GnRH receptors. *Trends In Endocrinology and Metabolism* 14 : 35-43. 2003.
- Millar RP and King JA. Synthesis, luteinizing hormone-releasing activity, and receptor binding of chicken hypothalamic luteinizing hormone-releasing hormone. *Endocrinology*, 113 : 1364-1369. 1983.
- Millar RP, Lu ZL, Pawson AJ, Flanagan CA, Morgan K and Maudsley SR. Gonadotropin-releasing hormone receptors. *Endocrine Reviews*, 25 : 235-275. 2004.
- Millar RP, Milton RC, Follett BK and King JA. Receptor binding and gonadotropin-releasing activity of a novel chicken gonadotropin-releasing hormone ([His5, Trp7, Tyr8] GnRH) and a D-Arg6 analog. *Endocrinology*, 119 : 224-231. 1986.
- Miyamoto K, Hasegawa Y, Igarashi M, Chino N, Sakakibara S, Kangawa K and Matsuo H. Evidence that chicken hypothalamic luteinizing hormone-releasing hormone is [Gln8]-LH-RH. *Life Sciences*, 32 : 1341-1347. 1983.
- Miyamoto K, Hasegawa Y, Minegishi T, Nomura M, Takahashi Y, Igarashi M, Kangawa K and Matsuo H. Isolation and characterization of chicken hypothalamic luteinizing hormone-releasing hormone. *Biochemical and Biophysical Research Communications*, 107 : 820-827. 1982.
- Miyamoto K, Hasegawa Y, Nomura M, Igarashi M, Kangawa K and Matsuo H. Identification of the second gonadotropin-releasing hormone in chicken hypothalamus : evidence that gonadotropin secretion is probably controlled by two distinct gonadotropin-releasing hormones in avian species. *Proceedings of the National Academy of Sciences of the United States of America*, 81 : 3874-3878. 1984.
- Morgan K, Conklin D, Pawson AJ, Sellar R, Ott TR and Millar RP. A transcriptionally active human type II gonadotropin-releasing hormone receptor gene homolog overlaps two genes in the antisense orientation on chromosome 1q.12. *Endocrinology*, 144 : 423-436. 2003.
- Murakami S, Seki T, Wakabayashi K and Arai Y. The ontogeny of luteinizing hormone-releasing hormone (LHRH) producing neurons in the chick embryo : possible evidence for migrating LHRH neurons from the olfactory epithelium expressing a highly polysialylated neural cell adhesion molecule. *Neuroscience Research*, 12 : 421-431. 1991.
- Neill JD, Duck LW, Sellers JC and Musgrove LC. A gonadotropin-releasing hormone (GnRH) receptor specific for GnRH II in primates. *Biochemical and Biophysical Research Communications*, 282 : 1012-1018. 2001.
- Norgren RB. Development of LHRH neurons in the avian embryo. *Annals of the New York Academy of Sciences*, 7 : 163-182. 1996.
- Norgren RB, Jr., Lippert J and Lehman MN. Luteinizing hormone-releasing hormone in the pigeon terminal nerve and olfactory bulb. *Neuroscience Letters*, 135 : 201-204. 1992.
- Oh DY, Wang L, Ahn RS, Park JY, Seong JY and Kwon HB. Differential G protein coupling preference of mammalian and nonmammalian gonadotropin-releasing hormone receptors. *Molecular and Cellular Endocrinology*, 205 : 89-98. 2003.
- Ottinger MA, Abdelnabi M, Li Q, Chen K, Thompson N, Harada N, Viglietti-Panzica C and Panzica GC. The Japanese quail : a model for studying reproductive aging of hypothalamic systems. *Experimental Gerontology*, 39 : 1679-1693. 2004.
- Pawson AJ, Katz A, Sun YM, Lopes J, Illing N, Millar RP

- and Davidson JS. Contrasting internalization kinetics of human and chicken gonadotropin-releasing hormone receptors mediated by C-terminal tail. *Journal of Endocrinology*, 156 : R9-12. 1998.
- Pawson AJ, Maudsley S, Morgan K, Davidson L, Naor Z and Millar RP. Inhibition of human type I gonadotropin-releasing hormone receptor (GnRHR) function by expression of a human type II GnRHR gene fragment. *Endocrinology*, 146 : 2639-2649. 2005.
- Pawson AJ, Maudsley SR, Lopes J, Katz AA, Sun YM, Davidson JS and Millar RP. Multiple determinants for rapid agonist-induced internalization of a nonmammalian gonadotropin-releasing hormone receptor : a putative palmitoylation site and threonine doublet within the carboxyl-terminal tail are critical. *Endocrinology*, 144 : 3860-3871. 2003.
- Perera AD and Follett BK. Photoperiodic induction in vitro : the dynamics of gonadotropin-releasing hormone release from hypothalamic explants of the Japanese quail. *Endocrinology*, 131 : 2898-2908. 1992.
- Proudman JA, Scanes CG, Johannsen SA, Berghman LR and Camp MJ. Comparison of the ability of the three endogenous GnRHs to stimulate release of follicle-stimulating hormone and luteinizing hormone in chickens. *Domestic Animal Endocrinology*, 2005.
- Proudman JA, Vandesande F and Berghman LR. Immunohistochemical evidence that follicle-stimulating hormone and luteinizing hormone reside in separate cells in the chicken pituitary. *Biology of Reproduction*, 60 : 1324-1328. 1999.
- Puebla-Osorio N, Proudman JA, Compton AE, Clements KE, Decuypere E, Vandesande F and Berghman LR. FSH- and LH-cells originate as separate cell populations and at different embryonic stages in the chicken embryo. *General and Comparative Endocrinology*, 127 : 242-248. 2002.
- Rozenboim I, Silsby JL, Tabibzadeh C, Pitts GR, Youngren OM and el Halawani ME. Hypothalamic and posterior pituitary content of vasoactive intestinal peptide and gonadotropin-releasing hormones I and II in the turkey hen. *Biology of Reproduction*, 49 : 622-626. 1993.
- Sakai H and Ishii S. Radioimmunoassay of avian FSH (Mikami S, Homma K and Wada M). pp. 125-133. Springer - Verlag. New York. 1983.
- Sellier N, Kisé Do Thi DX, Rousselot-Pailley D, Péczely P, De Rivier M and Guémené D. Effects of an LH-RH agonist on reproductive responses and endocrinological parameters in landais ganders. *Theriogenology*, 44 : 793-809. 1995.
- Senthilkumaran C, Peterson S, Taylor M and Bédécarrats GY. Use of a vascular access port for the measurement of pulsatile luteinizing hormone in old broiler breeders. *Poultry Science*, 2005. (in press).
- Seong JY, Wang L, Oh DY, Yun O, Maiti K, Li JH, Soh JM, Choi HS, Kim K, Vaudry H and Kwon HB. Ala/Thr (201) in extracellular loop 2 and Leu/Phe(290) in transmembrane domain 6 of type 1 frog gonadotropin-releasing hormone receptor confer differential ligand sensitivity and signal transduction. *Endocrinology*, 144 : 454-466. 2003.
- Sharp PJ. Photoperiodic regulation of seasonal breeding in birds. *Annals of the New York Academy of Sciences*, 1040 : 189-199. 2005.
- Sharp PJ, Dawson A and Lea RW. Control of luteinizing hormone and prolactin secretion in birds. *Comparative Biochemistry and Physiology. Part C : Pharmacology, Toxicology and Endocrinology*, 119 : 275-282. 1998.
- Sharp PJ, Dunn IC and Cerolini S. Neuroendocrine control of reduced persistence of egg-laying in domestic hens : evidence for the development of photorefractoriness. *Journal of Reproduction and Fertility*, 94 : 221-235. 1992.
- Sharp PJ, Dunn IC and Talbot RT. Sex differences in the LH responses to chicken LHRH-I and -II in the domestic fowl. *Journal of Endocrinology*, 115 : 323-331. 1987.
- Sharp PJ, Talbot RT, Main GM, Dunn IC, Fraser HM and Huskisson NS. Physiological roles of chicken LHRH-I and -II in the control of gonadotrophin release in the domestic chicken. *Journal of Endocrinology*, 124 : 291-299. 1990.
- Shimizu M and Bédécarrats GY. Identification of a novel pituitary specific chicken gonadotropin-releasing hormone receptor and its splice variants. *Biology of Reproduction* (in press).
- Somoza GM, Miranda LA, Strobl-Mazzulla P and Guilgur LG. Gonadotropin-releasing hormone (GnRH) : from fish to mammalian brains. *Cellular and Molecular Neurobiology*, 22 : 589-609. 2002.
- Sterling RJ and Sharp PJ. A comparison of the luteinizing hormone-releasing activities of synthetic chicken luteinizing hormone-releasing hormone (LH-RH), synthetic porcine LH-RH, and buserelin, an LH-RH analogue, in the domestic fowl. *General and Comparative Endocrinology*, 55 : 463-471. 1984.
- Stevenson TJ and Macdougall-Shackleton SA. Season- and age-related variation in neural cGnRH-I and cGnRH-II immunoreactivity in house sparrows (*Passer domesticus*). *General and Comparative Endocrinology*, 143 : 33-39. 2005.
- Stojilkovic SS, Reinhart J and Catt KJ. Gonadotropin-releasing hormone receptors : structure and signal transduction pathways. *Endocrine Reviews*, 15 : 462-499. 1994.
- Sullivan KA and Silverman AJ. The ontogeny of gonadotropin-releasing hormone neurons in the chick. *Neuroendocrinology*, 58 : 597-608. 1993.
- Sun YM, Dunn IC, Baines E, Talbot RT, Illing N, Millar RP and Sharp PJ. Distribution and regulation by oestrogen of fully processed and variant transcripts of gonadotropin releasing hormone I and gonadotropin releasing hormone receptor mRNAs in the male chicken. *Journal of Neuroendocrinology*, 13 : 37-49. 2001 a.
- Sun YM, Flanagan CA, Illing N, Ott TR, Sellar R, Fromme BJ, Hapgood J, Sharp P, Sealfon SC and Millar RP. A chicken gonadotropin-releasing hormone receptor that confers agonist activity to mammalian antagonists. Identification of D-Lys(6) in the ligand and extracellular loop two of the receptor as determinants. *Journal of Endocrinology*, 154 : 11-21. 2002.

- nal of Biological Chemistry, 276 : 7754–7761. 2001 b.
- Troskie BE, Hapgood JP, Millar RP and Illing N. Complementary deoxyribonucleic acid cloning, gene expression, and ligand selectivity of a novel gonadotropin-releasing hormone receptor expressed in the pituitary and mid-brain of *Xenopus laevis*. *Endocrinology*, 141 : 1764–1771. 2000.
- Tsutsui K, Saigoh E, Ukena K, Teranishi H, Fujisawa Y, Kikuchi M, Ishii S and Sharp PJ. A novel avian hypothalamic peptide inhibiting gonadotropin release. *Biochemical and biophysical research communications* 275 : 661–667. 2000.
- Urbanski HF. Episodic release of LH in gonadectomized male Japanese quail. *Journal of Endocrinology*, 100 : 209–212. 1984.
- Van Gils J, Absil P, Grauwels L, Moons L, Vandesande F and Balthazart J. Distribution of luteinizing hormone-releasing hormones I and II (LHRH-I and -II) in the quail and chicken brain as demonstrated with antibodies directed against synthetic peptides. *Journal of Comparative Neurology*, 334 : 304–323. 1993.
- Vizcarra JA, Kreider DL and Kirby JD. Episodic gonadotropin secretion in the mature fowl : serial blood sampling from unrestrained male broiler breeders (*Gallus domesticus*). *Biology of Reproduction*, 70 : 1798–1805. 2004.
- Wang C, Yun O, Maiti K, Oh DY, Kim KK, Chae CH, Lee CJ, Seong JY and Kwon HB. Position of Pro and Ser near Glu7.32 in the extracellular loop 3 of mammalian and nonmammalian gonadotropin-releasing hormone (GnRH) receptors is a critical determinant for differential ligand selectivity for mammalian GnRH and chicken GnRH-II. *Molecular Endocrinology*, 18 : 105–116. 2004.
- Wang L, Bogerd J, Choi HS, Seong JY, Soh JM, Chun SY, Blumenrohr M, Troskie BE, Millar RP, Yu WH, McCann SM and Kwon HB. Three distinct types of GnRH receptor characterized in the bullfrog. *Proceedings of the National Academy of Sciences of the United States of America*, 98 : 361–366. 2001.
- Wilson FE. Photoperiodism in american tree sparrows : role of the thyroid gland (Harvey S and Etches RJ). pp. 159–169. *Journal of Endocrinology Ltd. Bristol*. 1997.
- Wilson SC, Cunningham FJ, Chairil RA and Gladwell RT. Maturational changes in the LH response of domestic fowl to synthetic chicken LHRH-I and -II. *Journal of Endocrinology*, 123 : 311–318. 1989.
- Wilson SC and Sharp PJ. The effects of progesterone on oviposition and ovulation in the domestic fowl (*Gallus domesticus*). *British Poultry Science*, 17 : 163–173. 1976.
- Witkin JW, Dao D, Livne I, Dunn IC, Zhou XL, Pula K and Silverman AJ. Early expression of chicken gonadotropin-releasing hormone-1 in the developing chick. *Journal of Neuroendocrinology*, 15 : 865–870. 2003.
- Yang J, Long DW and Bacon WL. Changes in plasma concentrations of luteinizing hormone, progesterone, and testosterone in turkey hens during the ovulatory cycle. *General and Comparative Endocrinology*, 106 : 281–292. 1997.
- Yoshimura T. Molecular bases for seasonal reproduction in birds. *Journal of Poultry Science*, 41 : 251–258. 2004.
- Yoshimura T, Yasuo S, Watanabe M, Iigo M, Yamamura T, Hirunagi K and Ebihara S. Light-induced hormone conversion of T4 to T3 regulates photoperiodic response of gonads in birds. *Nature*, 426 : 178–181. 2003.
- Zhou W, Rodic V, Kitanovic S, Flanagan CA, Chi L, Weinstein H, Maayani S, Millar RP and Sealfon SC. A locus of the gonadotropin-releasing hormone receptor that differentiates agonist and antagonist binding sites. *Journal of Biological Chemistry*, 270 : 18853–18857. 1995.
- Zhou W and Sealfon SC. Structure of the mouse gonadotropin-releasing hormone receptor gene : variant transcripts generated by alternative processing. *DNA Cell Biology*, 13 : 605–614. 1994.