动物学报 52(Supplement): 174–177, 2006
*Acta Zoologica Sinica*

# S09-4 Molecular neuroendocrine mechanisms controlling photoperiodically regulated release of gonadotropin releasing hormone

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**Abstract** Change in photoperiod is a key proximal factor timing seasonal breeding at higher latitudes. Photoperiodic information is transduced in the neuroendocrine system to regulate the secretion of neuropeptides, gonadotropin releasing hormone-I (GnRH-I) and a vasoactive intestinal polypeptide, which respectively regulate the release of luteinizing hormone (LH) and prolactin. The initiation of photoinduced LH secretion is closely associated with an increase in hypothalamic GnRH mRNA, suggesting that an early event in the photoperiodic reproductive response is an increase in GnRH gene transcription. In many photoperiodic species, seasonal breeding is terminated by the development of absolute or relative photorefractoriness. Absolute photorefractory birds become unresponsive to very long days, including continuous light, while relative photorefractory birds become unresponsive to spring-like day lengths but remain responsive to very long days. The development of both absolute and relative photorefractoriness is associated with decreased plasma LH and increased plasma prolactin. Photorefractoriness develops for prolactin secretion in absolute photorefractory birds but not in relative photorefractory quail, *Coturnix*. Prolactin treatment depresses GnRH-I and LH beta mRNAs. It is suggested that the development of absolute and relative photorefractoriness may be initiated by increased plasma prolactin, and that increased prolactin maintains relative, but not absolute photorefractoriness.

**Key words** Gonadotropin releasing hormone (GnRH), Luteinizing hormone (LH), Photorefractoriness, Photoperiodism, Prolactin, Seasonal breeding

## 1 Introduction

Reproductive function is controlled by neuroendocrine responses to environmental stimuli that are integrated in the brain to increase or decrease the secretion of gonadotropins and prolactin from the anterior pituitary gland (Sharp et al., 1998). Of the many environmental factors that influence reproductive neuroendocrine function, the most extensively investigated is photoperiod (Nicholls at al., 1988; Sharp, 1996; Ball and Hahn, 1997; Hahn et al., 1997; Dawson, 1999; Dawson et al., 2001; Sharp and Blache, 2003). An example of the pattern of seasonal photoinduced changes in concentrations of plasma luteinizing hormone (LH) and prolactin in many photoperiodic birds is provided by the Svalbard ptarmigan, *Lagopus mutus hyperboreus* (Fig. 1A).

This bird breeds in the high Arctic where day length is continuous from May through August, and nights are continuous from November to January. Concentrations of plasma LH and prolactin increase when the threshold of critical day length increases above about 12 h, and remain high during the brief breeding season (Stokkan et al. 1988). A supplementary environmental factor — the availability of nests sites uncovered after the snow melts — determines the precise timing of onset of breeding in June/July (Stokkan et al., 1986). The breeding season is terminated by the development of absolute photorefractoriness, defined as insensitivity to photostimulatory day lengths, including continuous light (Nicholls et al., 1988; Sharp, 1996). The development of reproductive photorefractoriness is characterized by a steep fall in plasma LH when plasma prolactin values are maximal (Fig. 1A). After concentrations of plasma LH have begun to fall, plasma concentrations of prolactin also drop, demonstrating the development of absolute photorefractoriness for prolactin secretion too (Fig. 1A).

A less common type of annual breeding cycle is found in the European quail, *Coturnix coturnix coturnix* (Fig. 1B). Concentrations of plasma prolactin and LH increase after day length increases above about 12 h, and are high during the breeding season. Plasma LH begins to decrease in the fall when day-length is shortening but still above 15 h, followed by a drop in plasma prolactin in September/October when day length falls below 12 h. European quail therefore terminate breeding in the fall when day length is still longer than that required to stimulate breeding in the spring, but remain in breeding condition indefinitely if exposed to very long photoperiods (Boswell, pers comm.). This form of photorefractoriness is termed "relative" photorefractoriness. In the closely related and similarly responsive Japanese quail, *Coturnix coturnix japonica*, the development of relative photorefractoriness is demonstrated by the rapid decrease in plasma LH and gonadal regression that follows a reduction in the photoperiod to a 13 h day (Robinson and Follett, 1982; Follett and Pearce-Kelly, 1990).

## 2 The pathway for photoperiodic signal transduction

Light is detected by an extra retinal photoreceptor and the light signal is transmitted to a biological clock that measures day length using a circadian mechanism (Dawson, 1999; Dawson et al., 2001). Photoperiodic birds do not require the pineal gland nor the eyes for photoperiodic signal transduction (Wilson, 1991). Integrated photoperiodic information from the biological clock is transmitted to neurons synthesizing the neuropeptides, gonadotropin releasing hormone-I (GnRH-I) and vasoactive intestinal polypeptide (VIP), which respectively control the synthesis and secretion of gonadotropins and prolactin from the anterior pituitary gland (Sharp at al., 1998). The anatomical location of the extra retinal photoreceptor and biological clock is unknown, but a substantial body of information is available about the anatomical position of GnRH-I and VIP neurons (Teruyama and Beck, 2000, 2001). Photoperiodic information might be transduced via neural inputs to GnRH-I/VIP neurons at two sites: to their terminals in the median eminence and basal hypothalamus and/or to their cell bodies. It is predicted that neural inputs to terminals stimulate/inhibit GnRH-I/VIP release, while neural inputs to cell bodies stimulate/inhibit GnRH-I/VIP gene transcription.

## 3 Photoinduced GnRH-I gene transcription and GnRH-I release

The Japanese quail provides a model for determining whether photoinduced GnRH release is initiated by an increased release from GnRH terminals independently of a change in GnRH gene transcription. The Japanese quail and the domestic chicken are the only photoperiodic birds in which there are assays available to measure GnRH mRNA (Dunn et al., 1996; Dunn and Sharp, 1999; Baines, 2001). When sexually inactive photosensitive Japanese quail are transferred from short to long days, photoinduced GnRH release and LH secretion is initiated 20 h after dawn on the first long day (Perera and Follett, 1992). Increase plasma LH 20 h after dawn is associated with an increase in hypothalamic GnRH mRNA (Baines, 2001), suggesting that photoinduced reproductive function may be initiated by increased GnRH-I gene expression.

However there is also evidence that photoinduced GnRH release in quail is associated with increased neural activity in the median eminence and basal hypothalamus, suggesting that photoinduced GnRH-I release is also controlled at GnRH neuronal terminals (Meddle and Follett, 1997). This conclusion follows from the finding that the increase in LH release 20 h after dawn is preceded by an increase in the visible number of cells with increased fos immunoreactivity in the basal hypothalamus and median eminence. Fos is the protein product of an immediate early gene cfos; and Fos immunocytochemistry is widely used by neurobiologists to identify neurons and glial cells showing environmentally induced changes in gene transcriptional activity.

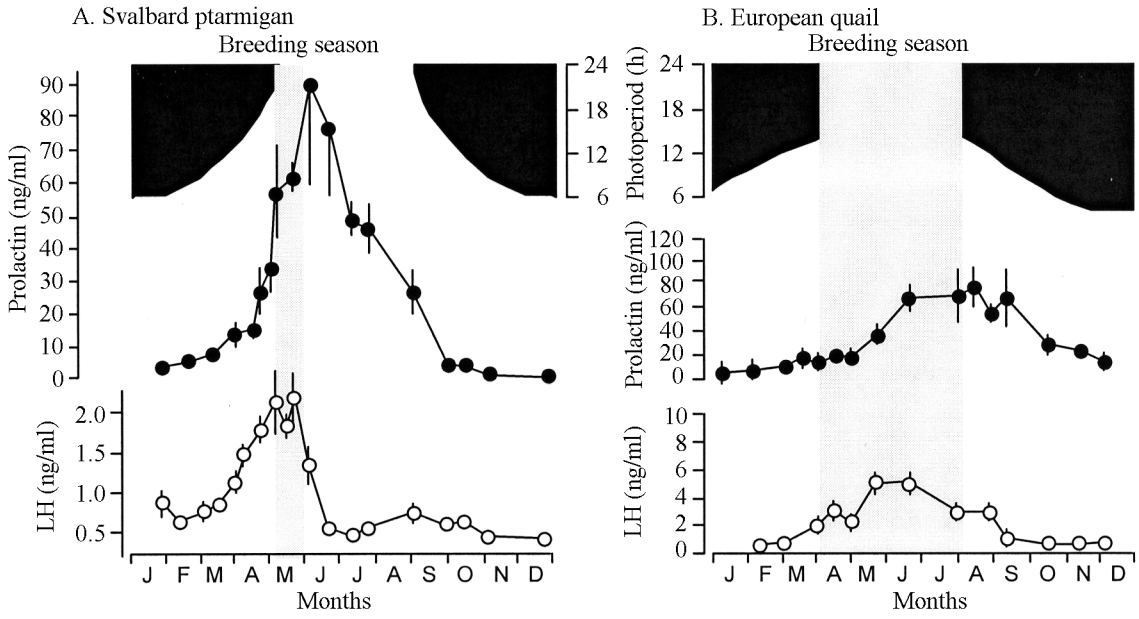


**Fig. 1 Changes in concentrations of plasma prolactin and luteinizing hormone (LH) in response to seasonal changes in photoperiod in birds which terminate seasonal breeding by A), the development of absolute photorefractoriness (Svalbard ptarmigan, *Lagopus mutus hyperboreus* breeding at 80°N — data from Stokkan et al., 1988) or by B), the development of relative photorefractoriness (European quail Coturnix coturnix coturnix breeding at 51°N — data from Boswell et al., 1993, 1995)** Observations on Svalbard ptarmigan were made on captive birds exposed to simulated seasonal changes in photoperiod: captive birds came into breeding condition earlier than in free-living birds, which breed in June (Stokkan et al., 1986). Observations on European quail were made on captive birds exposed to natural light: breeding occurred at the same time as in free-living birds (Cramp and Simmons, 1980).

## 4 Photo-inhibited GnRH-I gene transcription and GnRH-I release during development and maintenance of photorefractoriness

The development of reproductive photorefractoriness is thought to be a consequence of a progressive development of photoinduced inhibitory inputs to GnRH neurons (Parry and Goldsmith, 1993; Sharp, 1996; Dawson, 2001). If the development of photorefractoriness is initiated by an inhibition of GnRH release, it is predicted that photoinduced gonadal regression will be associated with maintained or increased basal hypothalamic GnRH peptide. Alternatively, it is also possible that the development of reproductive photorefractoriness is a consequence of decreased GnRH transcription and GnRH synthesis. If this hypothesis is correct, it is predicted that the onset of photorefractoriness will be preceded by a decrease in hypothalamic peptide and in GnRH mRNA.

The first of these hypotheses has been investigated by measuring changes in the amount of GnRH peptide in the basal hypothalamus in captive male European Starlings during the development of absolute photorefractoriness (Dawson et al., 2002). The development of absolute photorefractoriness, marked by rapid testicular regression after 4–6 weeks exposure to long day lengths, was associated with maintained basal hypothalamic GnRH peptide content. It is therefore concluded that the *initiation* of absolute photorefractoriness is caused by an inhibition of GnRH release. However, the *maintenance* of absolute photorefractoriness after complete testicular regression was associated with a 115-fold decrease in hypothalamic GnRH peptide content (Dawson et al., 2002). The maintenance of absolute photorefractorieness may therefore be a consequence of inhibited GnRH gene transcription. Methodology has not been developed to investigate this inference.

The availability of an assay to measure GnRH mRNA in Japanese quail makes it possible to determine whether the development of relative photorefractoriness in this species is associated with a change in hypothalamic GnRH mRNA. In a study reported by Baines (2001), the amount of GnRH mRNA in the hypothalamus of long term relatively photorefactory quail, in full breeding condition, was significantly lower than in breeding, fully photosensitive birds of the same age. The relatively photorefractory quail had been maintained on 18 h light per day for 67 weeks, while a fully photosensitive subgroup had been created by transferring birds first to 8 h light per day for six weeks to break relative photorefractoriness, and then returning them to 18 h light/day for 3 weeks to recover full breeding condition. No difference was found in the concentrations of plasma LH in the either quail group. This observation suggests that relative photorefractoriness associated with prolonged exposure to long days is maintained by decreased GnRH gene transcription. Further work is needed to confirm whether the development of relative photorefractoriness is associated with decreased GnRH mRNA.

## 5 Does prolactin play a role in the development of photorefractoriness?

There may be a causal relationship between the fall in plasma LH and increased plasma prolactin at the end of the breeding season in birds that terminate breeding by developing absolute or relative photorefractoriness (Figs. 1A and B). This hypothesis is consistent with the finding, in the starling, that immunosuppression of photoinduced prolactin release slows the rate of development of reproductive photorefractoriness (Dawson et al., 1998). The decrease in hypothalamic GnRH peptide content following the onset of photoinduced testicular regression is associated with peak values in plasma prolactin (Dawson et al., 2002). Is this association causal? Although no studies have been done in the starling, observations in the Japanese quail suggest that prolactin may depress GnRH neuronal function (Baines, 2001). Hypothalamic GnRH mRNA was significantly depressed in photosensitive Japanese quail injected twice daily for six days with prolactin, compared with vehicle-injected control birds, after transfer from short to long days. This observation suggests that increased plasma prolactin inhibits GnRH gene expression

The decrease in hypothalamic GnRH mRNA in quail treated with prolactin is associated with a decrease in plasma LH (Baines, 2001). This relationship may be causal. However, it could also be that prolactin acts directly at the level of the anterior pituitary gland to inhibit LH synthesis and release. This view is supported by observations *in vitro* using dispersed turkey pituitary cells (You et al., 1995). The addition of GnRH to turkey pituitary cell culture stimulates LH beta mRNA production and LH release. If prolactin is added with GnRH to the culture medium, the stimulatory effects of GnRH on LH beta mRNA and LH secretion are inhibited. Increased plasma prolactin at the end of the breeding season could therefore initiate relative or absolute photorefractoriness by acting through both the hypothalamus and the anterior pituitary gland to suppress GnRH and LH synthesis

## 6 Mechanisms responsible for relative and absolute photorefractoriness

It has been suggested that relative and absolute photorefractoriness differ in as much as relative refractoriness involves an inhibition of GnRH release without effect on GnRH synthesis and, by implication, GnRH gene expression, whereas absolute refractoriness involves inhibition of both GnRH release and synthesis (Hahn and Ball, 1995; Ball and Hahn, 1997; Dawson et al., 2001). The observation that GnRH mRNA is depressed in long term relatively photorefractory quail is not consistent with this hypothesis (Baines, 2001). A difference between the mechanisms underlying the two types of photorefractoriness may be found in the role of plasma prolactin in suppressing GnRH and LH beta gene expression. Increased plasma prolactin is associated with both develop-

ment and maintenance of relative photorefractoriness, at least in quail, but only with development in absolute photorefractoriness (Figs. 1A and B). Thus it is proposed that relative photorefractoriness is initiated and maintained by increased plasma prolactin, whereas absolute photorefractoriness is only initiated by it.

**Acknowledgements** This work was supported by a BBSRC Studentship and a Core Strategic Grant.

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