General and Comparative Endocrinology 190 (2013) 149–155

Contents lists available at SciVerse ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Photoreceptive oscillators within neurons of the premammillary nucleus (PMM) and seasonal reproduction in temperate zone birds

Sunantha Kosonsiriluk [a], Laura J. Mauro [a], Voravasa Chaiworakul [a], Yupaporn Chaiseha [b], Mohamed E. El Halawani [a,*]

[a] Department of Animal Science, University of Minnesota, St. Paul, MN 55108, USA
[b] School of Biology, Institute of Science, Suranaree University of Technology, Thailand

ARTICLE INFO

Article history:
Available online 27 February 2013

Keywords:
Birds
Clock genes
Dopamine
Melanopsin
Melatonin
Seasonal reproduction

ABSTRACT

The pathway for light transmission regulating the reproductive neuroendocrine system in temperate zone birds remains elusive. Based on the evidence provided from our studies with female turkeys, it is suggested that the circadian clock regulating reproductive seasonality is located in putatively photosensitive dopamine–melatonin (DA–MEL) neurons residing in the premammillary nucleus (PMM) of the caudal hypothalamus. Melanopsin is expressed by these neurons; a known photopigment which mediates light information pertaining to the entrainment of the clock. Exposure to a gonad stimulatory photoperiod enhances the activity of the DAergic system within DA–MEL neurons. DAergic activity encoding the light information is transmitted to the pars tuberalis, where thyroid-stimulating hormone, beta (TSHβ) cells reside, and induces the release of TSH. TSH stimulates tanycytes lining the base of the third ventricle and activates type 2 deiodinase in the ependymal which enhances triiodothyronine (T_3) synthesis. T_3 facilitates the release of gonadotropin-releasing hormone-I which stimulates luteinizing hormone/follicle stimulating hormone release and gonad recrudescence. These data taken together with the findings that clock genes are rhythmically expressed in the PMM where DA–MEL neurons are localized imply that endogenous oscillators containing photoreceptors within DA–MEL neurons are important in regulating the DA and MEL rhythms that drive the circadian cycle controlling seasonal reproduction.

Published by Elsevier Inc.

1. Introduction

The circadian clock regulating reproductive seasonality in temperate zone birds is localized in the mediobasal hypothalamus (MBH) (El Halawani et al., 2009; Kumar et al., 2010). This internal timing mechanism coordinates the reproductive neuroendocrine system to maintain synchrony with the environmental light–dark cycles. Light is the key cue used by temperate zone birds to synchronize seasonal reproductive activities. Novel findings from our laboratory have provided insight into the critical neuroanatomical structures and the neuroendocrine mechanism(s) involved in controlling the reproductive neuroendocrine system in the turkey, a temperate zone bird. These include the identification of photoreceptive dopaminergic (DAergic) neurons in the premammillary nucleus (PMM) of the turkey caudal hypothalamus (Thayananuphat et al., 2007a). PMM DA neurons reach threshold activation (as indicated by *c-fos* mRNA expression) when turkeys are exposed to light during the photoinducible phase and this coincides with the activation of gonadotropin-releasing hormone-I (GnRH-I) neurons, the up-regulation of *GnRH-I* mRNA expression, and the stimulation of the reproductive neuroendocrine system (Thayananuphat et al., 2007b).

2. Dopamine–melatonin (DA–MEL) neurons

In birds, light can reach the inner brain where extra-retinal photoreceptors are located integrating information with the circadian clock to regulate reproductive seasonality (Benoit, 1935). We have identified a group of neurons in the PMM; the most dorsal part of the MBH that co-express DA and MEL and are putatively photosensitive (El Halawani et al., 2009; Kang et al., 2007). MEL is a hormone that is a potent synchronizer of the oscillator and dictates the circadian entrainment in mammals (Dardente, 2012). DA–MEL neurons, in addition to exhibiting a daily endogenous dual-oscillation in DAergic/MELergic activities, express *c-fos* mRNA in association with the daily photoinducible phase of gonad growth (Thayananuphat et al., 2007a). Limited studies have addressed the contribution of these neurons to the regulation of the reproductive neuroendocrine system and their role in reproductive photoreception and photoperiodic time measurements (PTM) regulating reproductive seasonality.

* Corresponding author. Address: Department of Animal Science, University of Minnesota, 495 Animal Science/Veterinary Medicine, 1988 Fitch Avenue, St. Paul, MN 55108, USA. Fax: +1 612 625 2743.
E-mail address: elhal001@umn.edu (M.E. El Halawani).

0016-6480/$ - see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.ygcen.2013.02.015

A circadian mechanism driven by an intrinsic oscillator(s) is an established regulator of the avian reproductive cycle (Follett et al., 1985; Sharp and Follett, 1969). Since PMM DA–MEL neurons are putative sites of photoreception (Kang et al., 2010), then we would expect their activity to be influenced by such an oscillator. We show that expression of mRNAs encoding tyrosine hydroxylase (*TH*) and tryptophan hydroxylase 1 (*TPH1*), the first enzymes in the DA and MEL pathways, and in turn DAergic/MELergic activities cycle rhythmically and with opposite phases in PMM DA neurons of birds kept under a diurnal illumination cycle (12 h light/12 h dark; LD; Fig. 1). These findings are consistent with results showing that mRNAs and proteins of TPH1 and arylalkylamine N-acetyltransferase (AANAT; the enzyme which catalyzes the synthesis of MEL from serotonin; 5-HT), and TH, the first enzyme in DA synthesis, are also localized within these neurons. These results reveal an extra pineal and extra ocular sources of MEL which could be involved in reproductive regulation, since it is known that pinealectomized turkeys (Siopes and Underwood, 1987) as well as other avian species, including female Japanese quail (Underwood and Siopes, 1984) and house sparrow (Wilson, 1991), continue to have a normal reproductive cycle.

3. Melanopsin (OPN4) photopigment

Perhaps one of the important features of the MBH DA–MEL neurons is the expression of the photopigment OPN4 (Kang et al., 2010). Since its discovery and its localization to a subset of intrinsically photosensitive ganglion cells in the mammalian retina, this photopigment has been accepted as being responsible for entrainment to environmental cycles (Berson, 2007; Hankins et al., 2008). Turkeys are believed to detect light for circadian photoentrainment of the reproductive neuroendocrine function via DA–MEL neurons that express the photopigment OPN4 (Kang et al., 2010). And, extra-retinal photoreceptors contribute to reproductive functions through signaling via OPN4 containing DA–MEL neurons. Indeed, a light pulse provided during the photosensitive phase has been shown to activate GnRH-I neurons and up-regulated *GnRH-I* mRNA in these neurons, release luteinizing hormone (LH) and stimulate gonad growth (Kang et al., 2006; Thayananuphat et al., 2007b). These findings raise the possibility that turkey OPN4 protein in DA–MEL neurons may perform a light-sensing role in regulating seasonal reproduction in the turkey. Though, OPN4 may not be the only photopigment implicated in the photoperiodic response regulating reproductive seasonality as both opsin5 (OPN5) (Ohuchi et al., 2012) and vertebrate ancient opsin (VA opsin) (Davies et al., 2012) are also expressed in the avian brain.

Temperate zone birds have the ability to detect light for circadian photoentrainment of the reproductive neuroendocrine system in the absence of intact classical photoreceptors (Benoit, 1935). In addition, they have the capacity to anticipate changes to the day length with their internal biological clock, which permits them to coordinate their reproductive neuroendocrine activity for optimal reproductive performance. The synchrony between the light/dark cycle and circadian rhythms (i.e. circadian photoentrainment) requires the oscillator to receive light input from photoreceptive molecules (Menaker, 1968). These findings point to the OPN4-based irradiance detecting circuitry in photoreceptive DA–MEL neurons that drive the circadian reproductive neuroendocrine system (Kang et al., 2010).

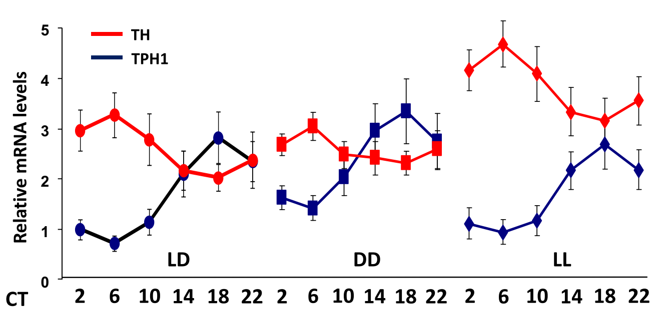

Fig. 1. Expression of mRNAs encoding tyrosine hydroxylase (TH) and tryptophan hydroxylase 1 (TPH1) cycle rhythmically with opposite phases in the premammillary nucleus under a diurnal illumination cycle 12 h light/12 h dark (LD), constant dark (DD), and constant light (LL). Modified from Kang et al. (2007).

The identification of the photopigment OPN4 in DA–MEL neurons suggests the first evidence for the long sought after extra-retinal photoreceptor regulating reproductive seasonality in birds (Fig. 2) (Kang et al., 2007). Electrophysiological recordings from OPN4-expressing ganglion cells, also known as intrinsically photosensitive retinal ganglion cells, and cells expressing OPN4 protein ectopically, clearly demonstrated that OPN4 is capable of responding to light and that it functions as a true photopigment (Bailes and Lucas, 2010; Schmidt et al., 2008). OPN4 plays a role in DA–MEL neurons photoreception. Photoreception and the full sensitivity of the reproductive neuroendocrine system to light require *OPN4* expression. Thus, under the conditions when birds are photorefractory and reproductive hormones reduced, *OPN4* expression is dramatically suppressed (Kang et al., 2010). Studies from our laboratory revealed that *OPN4* expression is rhythmic and confers photosensitivity to DA–MEL neurons, where the circadian clock is localized (Kang et al., 2007). These discoveries demonstrate that photoreception for photoentrainment occurs in DA–MEL neurons. Indeed, since the intrinsically photoreceptive DA–MEL neurons are first identified (Kang et al., 2007), it is clear that this photoreceptive system provides photic information to the avian reproductive neuroendocrine system (Kang et al., 2010). These findings taken together with the results that clock genes are being rhythmically expressed in DA–MEL neurons further support their involvement in photoperiodic time measurement regulating reproductive seasonality (Leclerc et al., 2010), thus making DA–MEL neurons the premier candidate for the specialized cells of irradiance detection and signaling to the reproductive neuroendocrine system.

In unpublished results from our laboratory, electrolytic lesion in the PMM where the intrinsically photosensitive DA–MEL neurons localized, circadian photoentrainment and light stimulation of the reproductive neuroendocrine system are severely impaired. It eliminates the induction of the early gene, *c-fos* (a marker for neuronal activation) in GnRH-I neurons, and the up-regulation of *GnRH-I* mRNA expression in response to a light pulse provided during the photosensitive phase for reproductive stimulation. These findings further indicate that extra-retinal photoreceptors contribute to reproductive function through signaling via OPN4-containing DA–MEL neurons. These results, taken together with the findings that cells expressing OPN4 protein ectopically are capable of responding to light (Bailes and Lucas, 2010; Schmidt et al., 2008), led to the suggestion that (1) OPN4 functions as a true photopigment in PMM DA–MEL neurons regulating reproductive seasonality and (2) in the absence of DA–MEL neurons and *OPN4* expression, no other photoreceptor is sufficient to provide signaling to the MBH. It would appear that the involvement of a second photopigment is therefore superfluous, OPN4 is sufficient to explain photoreception. However, it is worth noting that work reported in several papers (Foster et al., 1985; Halford et al., 2009; Nakane et al., 2010; Vigh et al., 1982; Yamashita et al., 2010) has demonstrated the expression of numerous photopigments in the avian brain and suggest their involvement in non-visual photoreception (Foster et al., 1994). This paradox implies that there must be redundancy in the mechanism by which photic information relayed to the MBH mediating the non-visual photoreception and reproductive seasonality.