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Deep Brain Photoreceptors and Photoperiodism in Vertebrates

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Abstract: Photoperiodism is an important adaptive phenomenon in various physiological parameters including reproduction to cope with seasonal changes. Involvement of extraretinal photoreceptors in the photoperiodism in non-mammalian vertebrates has been well established. In addition, circadian clock system is known to be involved in the photoperiodic time measurement. The pathway consists of light-input system, time measurement system (circadian clock), gonadotropin releasing hormone (GnRH) production in the hypothalamus, luteinizing hormone (LH) and follicle stimulating hormone (FSH) production in the pituitary, and final gonadal development.

Recently, several laboratories reported photopigments newly cloned in the pineal, eyes and deep brain in addition to already known visual pigments in the retina. These are pinopsin, parapinopsin, VA-opsin, melanopsin, etc. All these photopigments belong to the opsin family having retinal as the chromophore. However, the function of these photopigments remains unknown. I reviewed the studies on the location of the photopigments by immunocytochemistry. I also discussed the results on the action spectra for induction of gonadal development in relation with the location of the photoreceptors.

Various physiologically active substances distribute in the vertebrate brain. Such substances are GnRH, GnIH, neuropeptide Y, vasoactive intestinal peptide, c-Fos, galanin, neurosteroids, etc. I summarized the immunhistochemical studies on the distribution and the photoperiodic changes of these substances and discussed the route from the deep brain photoreceptor to GnRH cells.

Key words: photoperiodism, deep brain photoreceptors, vertebrates, immunohistochemisty

INTRODUCTION

Photoperiodism is an important adaptive phenomenon in various physiological parameters including reproduction to cope with seasonal changes. Involvement of extraretinal photoreceptors in the photoperiodism in non-mammalian vertebrates [1] has been well established since the pioneer work by Benoit [2]. The retinal photoreceptor acts for vision, while the extraretinal photoreceptors such as the pineal and the hypothalamus have the role as non-visual photoreceptors for photoperiodism. In addition, circadian clock system is known to be involved in the photoperiodic time measurement [3, 4]. One of the typical photoperiodic responses in animals is the photoperiodic-gonadal response. The pathway consists of light-input system, time measurement system (circadian clock), gonadotropin releasing hormone (GnRH) production in the hypothalamus, luteinizing hormone (LH) and follicle stimulating hormone (FSH) production in the pituitary, and final gonadal development (Fig. 1 and 2). In mammals (Fig. 1), light is perceived by the eye and the photic information is transferred to the suprachiasmatic nucleus (SCN), where the circadian clock resides and photoperiodic time measurement is performed. The integrated time information is transferred to the pineal, where melatonin is produced. Melatonin, in turn, exhibit photoperiodic response through GnRH pathway. On the other hand, in nonmammalian vertebrates (Fig. 2), each of the eye, pineal and deep brain has roles as the photoreceptor and clock [4]. In this report, I reviewed the studies on photoperiodism and deep brain photoreceptors in non-mammalian vertebrates and introduced recent investigations on the function of photopigments and the route from photoreception to GnRH production.

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INVOLVEMENT OF EXTRARETINAL AND EXTRAPINEAL PHOTORECEPTORS (DEEP BRAIN PHOTORECEPTORS) IN THE PHOTOPERIODIC GONADAL RESPONSE

Distinct gonadal development was induced by long photoperiods in blinded and pinealectomized Japanese quail [5], suggesting the existence of extraretinal and extrapineal photoreceptors. The extraretinal and extrapineal photoreceptors were shown to be in the hypothalamus by light guide experiments and implants of radioluminous paints [6, 7]. An action spectrum experiment in blinded

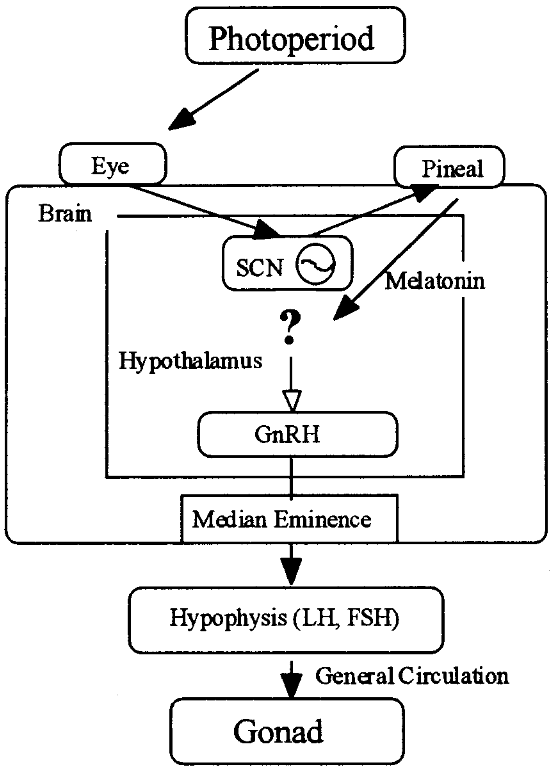

Fig. 1. A model illustrates the photoperiodic gonadal response in mammals.

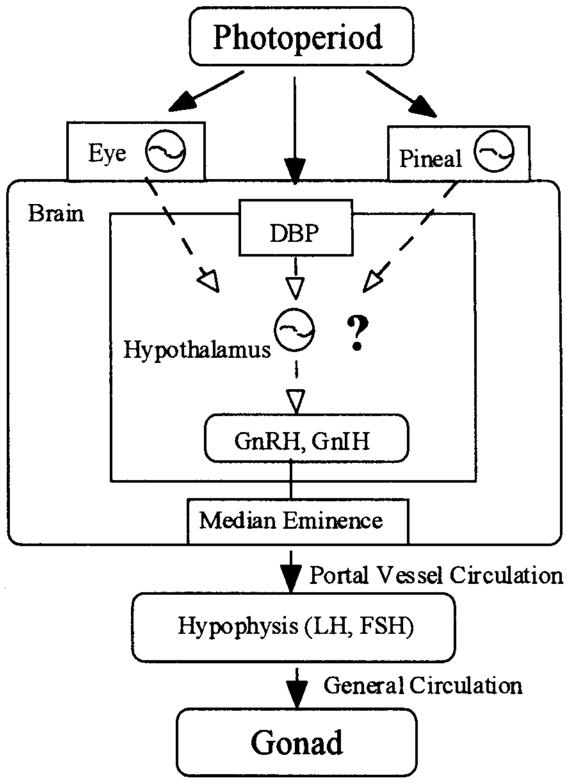

Fig. 2. A model illustrates the photoperiodic gonadal response in nonmammalian vertebrates.

and pinealectomized quail, in which the number of photons reaching to the hypothalamus was adjusted to be the same, revealed a peak at 500 nm [8]. Although the eyes and pineal with photoreceptors and circadian oscillators [4] might also be involved in the photoperiodic gonadal response of quail, extraretinal and extrapineal deep brain photoreceptors seem to have the most important role in the response.

Recently, several laboratories reported photoreceptive molecules newly cloned in the pineal, eyes and deep brain in addition to already known visual pigments in the retina. Pinopsin is the first extraretinal photopigment cloned [9]. As the deep brain photoreceptor, we found pinopsin in the toad hypothalamus [10]. Other deep brain photoreceptive molecules (rhodopsin, melanopsin, encephalopsin and VAL-opsin) and alpha subunit of rod transducin were reported to be localized in various regions of the brain of several species of vertebrates [11-15]. All these photopigments belong to the opsin family having retinal as the chromophore. Some of these photoreceptive molecules must be involved in the photoperiodic gonadal response. Judging from the peak action spectrum at 500 nm [8], rhodopsin and/or pinopsin are the most probable photoreceptive molecules in photoperiodic gonadal response in Japanese quail.

c-FOS EXPRESSION INDUCED BY LONG-DAY STIMULATION

Meddle and Follett [16] previously identified two hypothalamic regions (Infundibular nucleus and median eminence) where c-Fos-like immunoreactivities were induced in response to a long day. We used a different anti-c-Fos antibody recognizing a region from Lys^{128} to Ala^{152} of human c-Fos, and found many c-Fos-like immunoreactive nuclei localizing within nucleus anterior medialis hypothalami and nucleus periventricularis hypothalami [17]. We determined the whole coding sequence of quail c-Fos and compared the antigenic sequences of the two antibodies with the amino acid sequence of quail c-Fos. It was found that our antibody would recognize quail c-Fos more specifically than that used by Meddle and Follett [16]. We could confirm that the long-day stimulation induced c-Fos expression although the regions with c-Fos-like immunoreactive nuclei were slightly different probably due to the differences in epitope. We also found E-box like sequences and cyclic AMP responsive elements (CRE) in the upstream of c-fos gene. This suggest that c-Fos production is regulated by circadian clock system and light [18].

PHOTOPERIODIC CHANGES IN THE NEUROPEPTIDES

Various physiologically active neuropeptides distribute in the vertebrate brain. Such neuropeptides are gonadotropin releasing hormone (GnRH), gonadotropin inhibiting hormone (GnIH), neuropeptide Y (NPY), vasopressin, vasoactive intestinal peptide (VIP) and galanin. We investigated immunocytochemically the distribution of serotonin-, galanin-, VIP-, vasopressin- and NPY-immunoreactive (IR) neurons in the hypotghalamus of Japanese quail [19]. We also tried to observe whether there are any effects of photoperiods on the numbers of these immunoreactive cells. The serotonin-IR cells were located in the paraventricular organ (PVO) and infundibular nucleus (IF), and the number of these cells under short photoperiod was less in the dark period than in the light period. Galanin-IR cells were also found in PVO and ventral region of IF, and the number of these cells was significantly greater in short photoperiod than in long photoperiod. The galanin-, VIP-, AVP- and NPY-IR neurons innervated the serotonin-IR cells in PVO and IF. Since interaction between the VIP and GnRH has been reported in Japanese quail [20], above observations suggest that the neurons, especially serotonin- and galanin-IR cells, participate in the photoperiodic gonadal response of Japanese quail.

SEASONAL AND PHOTOPERIODIC CHANGES IN NEUROSTEROIDS

It is now well established that the vertebrate brain synthesizes steroids from cholesterol [21]. However, the function of the neurosteroids in the brain remains unclear. Therefore, we tried to investigate the relation of neurosteroids and the photoperiodicity in a seasonally breeding amphibian, *Cynops pyrrhogaster* (Inai et al., in preparation). Cytochrome P450 side-chain cleavage enzyme (P450scc) and 3β-hydroxysteroid dehydrogenase /D^5-D^4-isomerase (3βHSD) were immunocytochemically detected in the preoptic area of the newt brain. The pregnenolone and progesterone concentrations in the brain showed marked changes during annual breeding cycle and a maximal level in August, independent of the plasma steroid levels that were always low. Progesterone levels in the brain also showed a peak in April. Both pregnenolone and progesterone concentrations in the brain were significantly

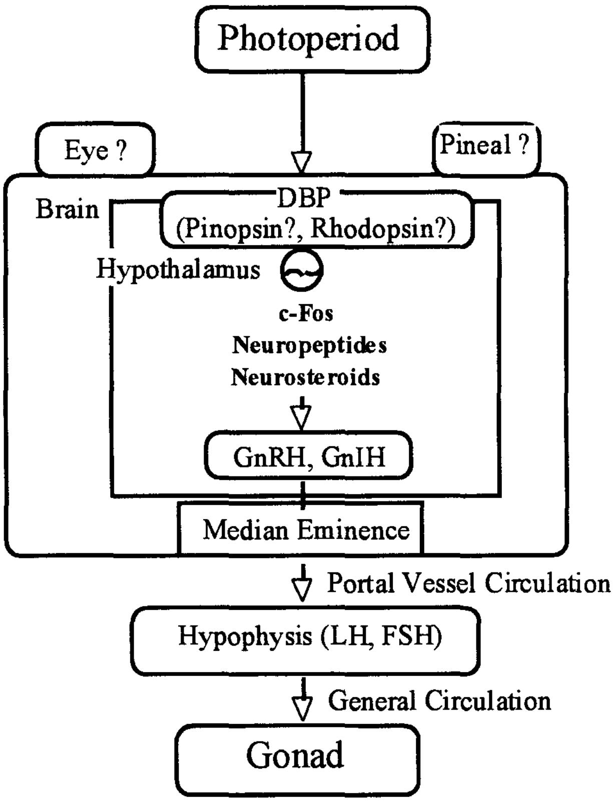

Fig. 3. A model illustrating the involvement of c-Fos, neuropeptides and nurosteroids in the photoperiodic gonadal response.

higher in the long day group than in the short day group, whereas no significant effects of different ambient temperatures on neurosteroid levels were detected. Photoperiod may be a more important environmental factor than temperature for the regulation of neurosteroids levels in the newt brain. However, the role of neurosteroids in the photoperiodic gonadal response remains to be studied in future.

CONCLUSIONS

As it is shown in Fig. 3, photoperiodic information is received by deep brain photoreceptors in nonmammalian vertebrates. Various photoreceptive molecules including pinopsin and rhodopsin have been reported as the photopigments in deep brain photoreceptors, and suggested to be the major photopigments involved in photoperiodic gonadal response. The information is transferred to GnRH and GnIH system in the hypothalamus via probably c-Fos system, which has E-box and CRE in the upstream regulatory region [18]. In the hypothalamus, neuropeptides such as serotonin, galanin and VIP, and neurosteroids such as pregnenolone and progesterone are suggested to regulate GnRH and GnIH system in birds and lower vertebrates.

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