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POLYCLONAL ANTIBODY

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Catalog No. LSL-LB-5509

Anti Rhodopsin

Product type	Primary antibodies
Host	Rabbit
Source	Serum
Form	Liquid This product does not contain preservatives such as NaN3.
Volume	100µl
Concentration	
Specificity	Rhodopsin
Antigen	Octopus Retina
Isotype	

Application notes	ELISA, WB, IHC **Recommended use** According to the ELISA assay, results are positive for dilutions up to 500,000 fold. Immunohistochemistry on Frozen or Paraffin section **Recommended dilutions** Immunofluorescence; More than 1/500 (recommended: 1/200～1/10,000) Optimal dilutions/concentrations should be determined by the end user. **Staining Pattern** Appears as band corresponding Rhodopsin by Western blotting.
Cross reactivity	Cross react with Octopus, Calamary and frog. Low cross react with chicken and mammalian. Cross reaction will differ between species.
Storage	Store below -20°C (below -70°C for prolonged storage). After thawing, store in small aliquots in sealable vials and store below -70°C. To prevent degradation from repeated thawing, store the antiserum between 0 to 4°C after second thawing. Stable for three years at -70°C
References	

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Possible involvement of light regulated gonadotropin-releasing hormone neurons in biological clock for reproduction in the cerebral ganglion of the ascidian, *Halocynthia roretzi*

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Abstract

Since ascidians, a primitive chordate, spawn at a fixed latency after sunrise, light must regulate a biological clock for reproduction in the ascidians. A retinal protein found in the cerebral ganglion of the ascidian is a candidate for the photoreceptor that might drives the change in gonadal activity via the gonadotropin-releasing hormone (GnRH) system. Photoresponses of the cerebral ganglion of ascidian, *Halocynthia roretzi*, were examined and two light-evoked responses recorded extracellularly, a light-evoked slow potential and light inhibition of high frequency spontaneous discharges. These results suggest that pacemaker signals of GnRH neurons might be regulated by photoreceptor activation. Immunohistochemical studies showed photoreceptor cells located close to the GnRH neurons and thus the photosignal might proceed from photoreceptor cell to GnRH neuron intercellularly. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Biological clock; Gonadotropin-releasing hormone; Retinal protein; Ascidian; Reproduction

The predominant Zeitgeber for a biological clock for reproduction is photoperiod that may drive annual changes in gonadal activity of the preoptic-hypothalamic gonadotropin-releasing hormone (GnRH) system [1]. Since the period of most productive biological clock is annual, it is difficult to accumulate reproducible data for behavioral and physiological studies. The ascidian, a primitive chordate, is an ideal animal to study the molecular mechanism for the biological clocks for reproduction, because ascidians spawn at a fixed latency period after sunrise everyday in the spawning season [5,7,12,16]. These phenomena can be reproduced in the laboratory. For example, a noon type of *Halocynthia roretzi* spawns 6 h after exposure to light following 6 h of darkness at 18°C in the laboratory whenever started. Two other types, morning and evening types spawn 4 and 12 h after sunrise, respectively, [12]. These results suggest that the biological clock is initiated by light and the latency time is specific to the type of *H. roretzi*.

An encephalic photoreceptor has been found in the deep part of the brain and a retinal protein in the hypothalamus is essential for seasonal reproduction [18]. It has been suggested that a photoreceptor that controls the spawning exists in the cerebral ganglion of the ascidian [4,8]. Recently, retinal proteins in the cerebral ganglion of an ascidian, *H. roretzi*, were successfully visualized and their localizations were determined by the retinal protein imaging method [8]. Retinal proteins in the cerebral ganglion of the *H. roretzi*, were localized mainly at the surface of the anterodorsal root and the posterodorsal root ganglia. In cross sections along the anteroposterior axis of the cerebral ganglion, cells bearing retinal proteins were found in the peripheral cellular cortex.

GnRH immunoreactive neurons were shown to exist in the cerebral ganglion of ascidians [2,6,11,15]. Since the GnRH of the vertebrate brain plays a pivotal role as a neurotransmitter or neuromodulator facilitating reproductive behaviors [3,10], regulation of GnRH neurons by light may be important to drive the biological clock for reproduction. It was demonstrated that injection of GnRH into the body of ascidians induced spawning, especially injection into the lumen of gonoduct was most effective [14]. Since most of GnRH neurons exhibit a spontaneous beating discharge pattern [10], it could be potentially involved in a pacemaker for biological clock.

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In this paper, we report two light-evoked responses recorded extracellularly, a light-evoked slow potential and light inhibition of high frequency spontaneous discharges in the cerebral ganglion of the ascidian, *H. roretzi*. Localization of retinal protein immunoreactive cells (photoreceptor cells) and GnRH immunoreactive cells in the cerebral ganglion were determined by immunohistochemical method.

Ascidian, *H. roretzi*, was purchased from fishermen in Sameura Bay at Miyagi, Japan and animals were maintained in running artificial seawater (Marine Art BR, Senjyu, Osaka, Japan) at 8–10°C in our laboratory. The cerebral ganglion, which is located between the base of the oral and the atrial siphons of *H. roretzi*, was isolated surgically and the peripheral muscles and connective tissues removed. The isolated cerebral ganglion was fixed by insect pins at the bottom of Silgard resin of the chamber. Two types of recording electrodes were used. Tungsten electrode was used for recording of slow potentials and a glass electrode for spike activities. The indifferent electrode of an $Ag–AgCl_2$ plate was fixed at the chamber. Responses to light were led to an oscilloscope and a pen recorder through DC or AC amplifiers and stored on a DATA recorder simultaneously.

When light (2.0 mW/cm^2) from a halogen lamp was irradiated on the surface of a cerebral ganglion, a slow potential with the recording electrode side positive was recorded as shown in Fig. 1a. Superimposed recordings indicate responses for different duration but an equal intensity of light. This positive response lasted during the light illumination. The amplitude of responses increased with increase of the intensity or duration of light. The response for stimulation of the duration less than 2 s did not reach the maximum amplitude even if when strong light was used. The amplitude of the response increased when the electrode was touched on the surface of the anterodorsal and posterodorsal roots of the cerebral ganglion, where retinal bearing cells were shown to locate abundantly in our previous paper [8]. The light-evoked positive slow wave is a field potential which is similar to an electroretinogram (ERG) in the retina. The positive slow extracellular potential means that with intracellular recording the cell would be hyperpolarize.

A second type of light-evoked response was recorded with a glass electrode, filled with 2 M KCl and having a tip resistance of 10–20 MΩ in artificial seawater. When the electrode was inserted in the cerebral ganglion in the dark, high frequency spontaneous discharges were recorded as shown in Fig. 1b. When the cerebral ganglion was illuminated by light, the spontaneous discharges were completely inhibited. Recently Tatsuki and Oka [16] recorded several types of responses to light from neurons of cerebral ganglion of *Ciona savignyi* using intracellular recordings. One of their response types was similar to the present results – light inhibition of a spontaneous discharge. The origin of these spontaneous discharges is not known. The cerebral ganglion of an ascidian contains an extremely small number of neurons compared with those of invertebrate ganglia [13] and GnRH-immunoreactive neurons were found in the cerebral ganglion of *Ciona intestinalis* [6,15]. It has been demonstrated in a teleost brain that GnRH neurons show regular spontaneous discharges [9]. Thus, it may be possible that the extracellular recording of spontaneous activity of the ganglion of *H. roretzi* is generated from GnRH neurons.

The present results suggest that two different responses to light were recorded from the cerebral ganglion of *H. roretzi*, a slow potential generated from the photoreceptor cells and spontaneous discharges from the GnRH neurons, respectively.

To know the relationship between photoreceptor cells and GnRH neurons, we examined the localization of retinal protein bearing cells and GnRH bearing cells in the cerebral ganglion by immunohistochemistry. Immunohistochemical procedure of Terakado et al. [13] was followed. The cerebral ganglion was fixed with formaldehyde solution. After the tissues were dehydrated through graded alcohol, they were embedded in paraffin and sectioned at 6 μm. The sections were treated with the primary antiserum (antibody against octopus rhodopsin, ×5000 (PSL, Tokyo, Japan) and antibody against GnRH, ×5000 (Anti-LHRH; Biogenesis LTD, USA). Immunoreactivity was visualized by two different methods, the ABC-Kit (Vector Lab) and 3,3′-diaminobenzidine (DAB) reaction and second antibodies

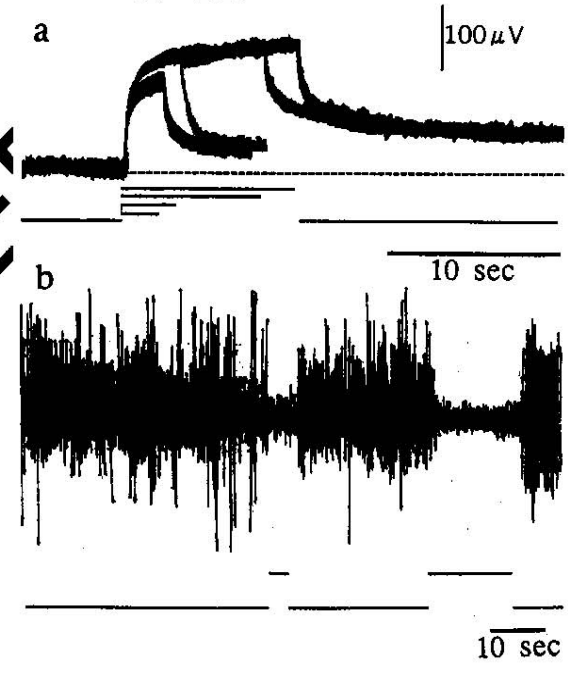

Fig. 1. Light-evoked responses were recorded extracellularly from the cerebral ganglion of *H. roretzi*. (a) The light-evoked slow potential which was recorded with tungsten electrode. Superimposed recordings indicate responses for different duration but an equal intensity of light. Lower traces show duration of light, which changed height in order to indicate length of light. The response for short flash of light less than 2 s does not reach to the maximum amplitude. Upward deflection indicates onset of light in Fig. 1a,b. As for the characteristic of a response for long light stimulation, long-lasting potential which was continued for about 1 min after cessation of light was observed.

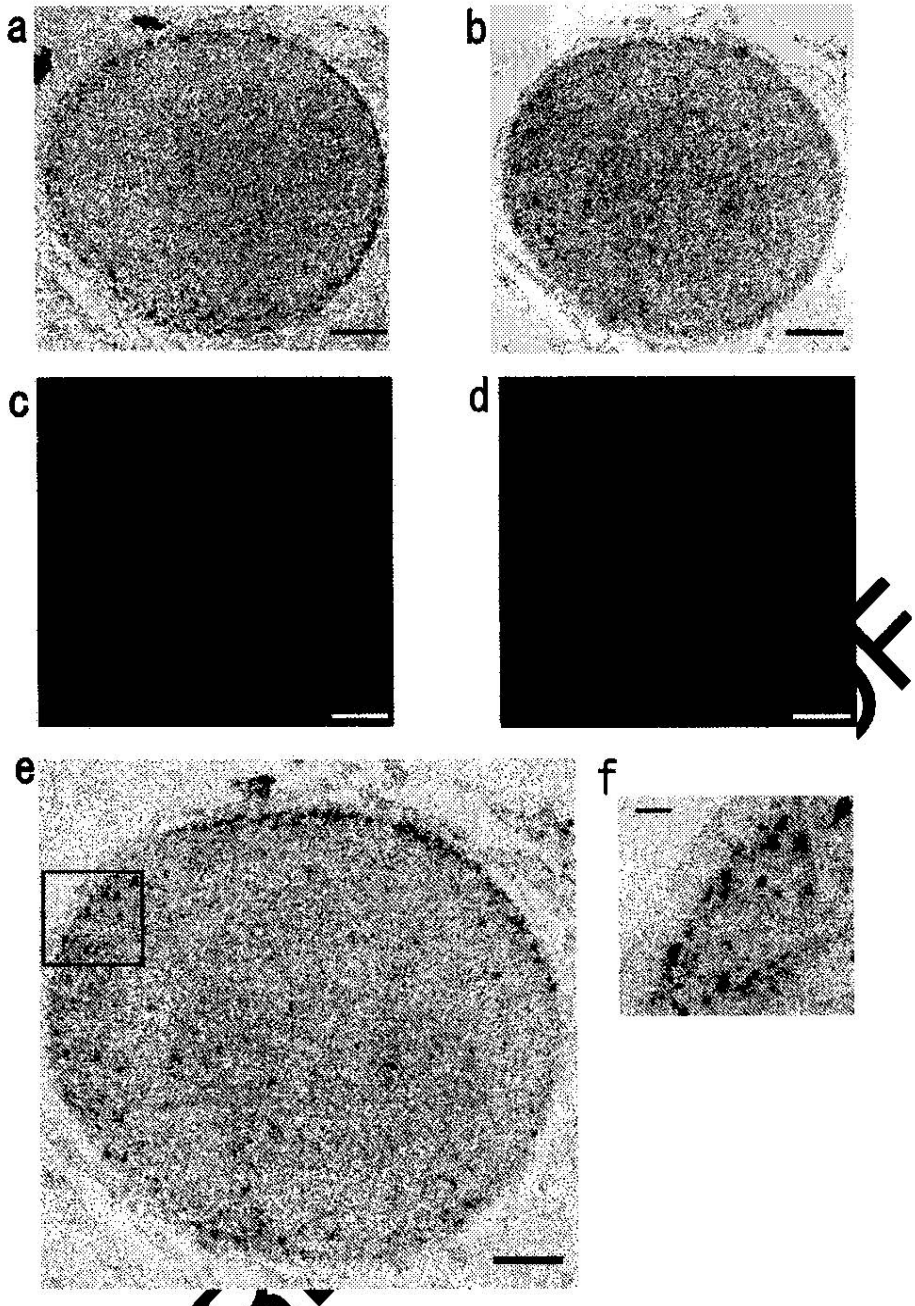

Fig. 2. The figure shows the cross section along the anteroposterio axis of the cerebral ganglion. The immunopositive cells to the polyclonal antibody against octopus rhodopsin were visualized by DAB (a) and FITC (c). The cross sections of the cerebral ganglion reacted with antibody against GnRH visualized by DAB (b) and FITC (d), respectively. Double staining of the section by antibody against octopus rhodopsin and antibody against GnRH. The immunopositive cells to antibody against octopus rhodopsin were visualized by FITC (b) and the immunopositive cells to antibody against GnRH were visualized by DAB (c) in the same section. Panels b and c were superimposed to Panel e. Bar, 100 μm. Panel f was a part of Panel e. Bar, 20 μm.

labeled by fluorescein isothiocyanate (FITC). To test the specificity of the antibody, control sections were incubated in antibody preabsorbed with antigen (octopus rhodopsin, 5 μg/ml or human GnRH, 2.5 μg/ml). Omission of primary antibody was also performed. These procedures completely blocked specific immunostaining (data are not shown).

Immunoblot analysis of the extract of the cerebral ganglion was performed with eight different antibodies against rhodopsins, three polyclonal antibodies against bovine, frog and octopus rhodopsins, and five monoclonal antibodies against octopus rhodopsin. We found that only polyclonal antibody against octopus rhodopsin was immunopositive to the 32 kDa protein in the extract of the cerebral ganglion (paper is in preparation).

Fig. 2 shows the cross section along the anteroposterior axis of the cerebral ganglion. The immunopositive cells to the polyclonal antibody against octopus rhodopsin were visualized by DAB (Fig. 2a) and FITC (Fig. 2c). Both panels

show that immunopositive cells localized at the peripheral cellular cortex. The localization of the rhodopsin immunopositive cells in the cerebral ganglion was essentially the same as those shown by the retinal protein imaging method [8]. These results suggest retinal protein bearing cells existed in the peripheral cellular cortex of the cerebral ganglion of the ascidian.

Next, we examined the localization of GnRH-immunoreactive neurons in the cerebral ganglion. Fig. 2b,d show the cross sections of the cerebral ganglion reacted with antibody against GnRH visualized by DAB and FITC, respectively. GnRH-immunoreactive cell bodies and fibers were distributed in the entire part of the cerebral ganglion. However, the cell bodies of the GnRH neuron densely existed at the peripheral cellular cortex and the neurites from the cell bodies extended to the central neuropile. Thus the retinal bearing cells and the GnRH neurons existed in the same area, the peripheral cellular cortex, of the cerebral ganglion.

In order to examine whether the retinal bearing cells and the GnRH neurons are the same or not, double staining of the section by anti-GnRH and anti-rhodopsin antibodies was performed. The sections of the cerebral ganglion were first treated with the antibody against GnRH and stained by DAB, as described before. Then we applied the antibody against octopus rhodopsin to these sections, and washed with phosphate-buffered saline (PBS) and treated with second antibodies that were labeled FITC. The fluorescence micrograph of rhodopsin-immunoreactive cells stained by FITC (Fig. 2c) and the light micrograph of GnRH-immunoreactive neurons stained by DAB (Fig. 2b) were superimposed as shown in Fig. 2e,f. These results showed that the both types of neurons do not overlap with each other and retinal proteins are not present in the GnRH neuron, but are in the photoreceptor cells located close to the GnRH neuron in the peripheral cellular cortex.

The present results suggest that light activated retinal protein leads to hyperpolarization of the photoreceptor cell. It might be possible that a persistent transmitter release, which persistently facilitates the GnRH pacemaker potentials, is disfacilitated by the photoreceptor hyperpolarization. Thus, the GnRH neurons might control the reproduction system [14].

0. Uncited References

[17].

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L S L

カタログNo. LB－5509

Anti (octopus) Rhodopsin

Ⅰ. 内容

lot No. 842041

免疫動物	ウサギ
性状・包装ｻｲｽﾞ	全血清・１００μℓ
力価	ELISA で500,000 倍希釈まで陽性
抗原由来	ミズダコ・網膜
種間交差	タコ・イカ・カエルと交差＊
特徴	網膜抽出物に対してWestern blottingでRhodopsinに対応するバンドと反応。組織染色で網膜の桿細胞(ﾛﾄﾞﾌﾟｼﾝの存在部位）が染色される。
標準希釈率	蛍光抗体法で１：５００以上 (1/200 ～1/10,000)

＊種によって交差性が異なります、希釈率等充分検討下さい。ニワトリ及びホ乳動物との交差性は低いと思われる。
（ ）内は推薦希釈倍率

Ⅱ. 保存上の注意

－２０℃以下（長期間の場合は－７０℃以下）で凍結して下さい。
解凍後は密栓のできる小型容器に研究の規模に応じて少量づつ分注し、－７０℃以下で保存して下さい。
凍結融解の繰り返しによる力価の低下を避ける為、再解凍後の抗血清は０～４℃に保ち操作・保存して下さい。

Ⅲ. 安定性

－７０℃で３年間安定。
但し、NaN_3 等の防腐剤は入っていません。

製造元

株式会社 エル・エス・エル

総発売元

コスモ・バイオ株式会社