Comparative Biochemistry and Physiology, Part A xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Octopamine cyclic release and its modulation of visual sensitivity in crayfish

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ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form 30 August 2016
Accepted 30 August 2016
Available online xxxx

Keywords:
Octopamine
Vision
Modulation
Crustaceans
Diurnal rhythms
Electroretinogram
Phentolamine
Procambarus clarkii.

ABSTRACT

The biogenic amine octopamine (OA) modulates invertebrate behavior by changing neuronal responses from sensory inputs to motor outputs. However, the OA modulation of visual sensitivity and its possible coupling to diurnal cycles remains unexplored. Here we studied the diurnal variations in the OA levels in the hemolymph of the crayfish *Procambarus clarkii*, its release from the structures in the eyestalk and its modulation of the retinal light sensitivity. The hemolymph concentration of OA and its amino acid precursor tyrosine was measured by high-resolution liquid chromatography; OA varied along the 24-h cycle. The peak value appeared about 2 h before the light offset which preceded the peak locomotor activity. OA was found in every structure of the eyestalk but displayed higher levels in the retina–*lamina ganglionaris*. Moreover, OA was released from isolated eyestalks at a rate of 92 nmol/eyestalk/min and a calcium-dependent release was evoked by incubation in a high potassium solution. OA injected into dark-adapted crayfish or applied to the isolated retina at concentrations of 1, 10 and 100 μM produced a proportionally increasing reduction in the amplitude of the photoreceptor light responses. These OA concentrations did not affect the position of the visual accessory pigments. Our results suggest that OA release in the crayfish eyestalk is coupled to the 24-h cycle to regulate the diurnal reduction of the photoreceptor sensitivity and to favor the expression of exploratory locomotion during the dark phase of the circadian cycle.

1. Introduction

The environmental changes along the 24-h period impose major adaptive challenges to animal behavior. Among these environmental stimuli, light has a significant importance for producing reflex responses and also for synchronizing the physiological activity of the circadian system (Fernández de Miguel and Aréchiga, 1992, 1994). The changes in the light intensity along a day may expand within approximately 9 logarithmic intensity units (Nilsson, 2009). Thus, the visual system must respond to these challenges by adapting its sensitivity along the day. An interesting example of the behavioral responses to light is provided by crustacean since their out-of-burrow activity is coupled to the moments of dim lights in such a manner that animals living in shallow waters, where the light levels are high, display their activity during the night; animals of the same species living at intermediate depths display nictemeral activity, and animals in deep waters receiving low intensity levels may display diurnal activity (Aréchiga and Atkinson, 1975). This light-induced reflex activity is also coupled to the circadian modulation through the interplay of several pacemakers synchronized by light (Page and Larimer, 1972; Fernández de Miguel and Aréchiga, 1992; Aréchiga et al., 1993; Rodríguez-Sosa et al., 2008; Strauss and Dircksen, 2010; Mendoza-Vargas et al., 2015), with food being an alternative synchronizing stimulus (Fernández de Miguel and Aréchiga, 1994). Within this context, the search for internal couplers of light with reflex or circadian behaviors has led to the discovery of multiple modulators of the visual responses of crustacean. A well-known example is that of 5-hydroxytryptamine (5-HT, serotonin), which in crayfish increases the visual sensitivity of photoreceptors and adapts an accessory visual pigment to darkness (Rodríguez-Sosa and Aréchiga, 1982; Aréchiga et al., 1990; Picones and Aréchiga, 1990).

Octopamine (OA) in invertebrates modulates social responses and other behaviors from sensory inputs to motor outputs, evoking the effects opposite to those of serotonin (Kravitz, 1988; Livingstone et al., 1980; Roeder, 1999; Roeder et al., 2003; Pedetta et al., 2010; Verlinden et al., 2010; Christie, 2011; Momohara et al., 2013). Moreover, in the crab, *Limulus polyphemus* OA may be linked to a circadian oscillator since it increases the visual response of the lateral eye as an

Abbreviations: OA, octopamine; Tyr, tyrosine; 5-HT, 5-hydroxytryptamine, serotonin; ES, eyestalk; R–LG, retina–*lamina ganglionaris*; ME, *medulla externa*; MI, *medulla interna*; MT, *medulla terminalis*; SG, sinus gland; ERG, electroretinogram.

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http://dx.doi.org/10.1016/j.cbpa.2016.08.032
1095-6433/

endogenous circadian pacemaker would do (Battelle, 2013). OA is released into the hemolymph from the pericardial organ of lobsters (Evans et al., 1976) and in the central nervous system and hemolymph of the crayfish *Pacifastacus leniusculus*, OA reaches a concentration higher than that of serotonin (Elofsson et al., 1982), thus suggesting its role in modulating the daily activity that is linked to circadian pacemakers. For these reasons, here we examined the possible role of OA as a modulator of the visual input in the crayfish and its correlation with its diurnal variations.

We measured the effects of OA on the massive responses to light of retinal photoreceptors in whole crayfish or in isolated retina–*lamina ganglionaris* (R–LG). The content of OA and its amino acid precursor tyrosine (Tyr) was quantified from different regions of the eyestalk. OA release was quantified from isolated eyestalks and its concentration in the hemolymph was measured along the 24-h cycle in correlation with the 24-h locomotor rhythm.

2. Materials and methods

2.1. Animals

We used 126 adult crayfish *Procambarus clarkii* of either sex with a weight of 20–30 g and a length of 10–12 cm from the *rostrum* to *telson*. Animals were supplied by a local provider and were kept in the laboratory for two weeks before the experiments, in aerated water containers, with a program of 12:12 h of light–dark cycles, and free access to vegetables and dried fish as food. The light intensity during the illumination period was 200 lx. Most experiments were made between 11:00 and 14:00 h, except for the measurements of the OA daily variations. Animal care was in accordance with the policies of the Society for Neuroscience (2016).

2.2. Electrophysiological recordings of visual responses

The electroretinogram (ERG), the visual response from retinal photoreceptors caused by photo-stimulation, was recorded from 12 crayfish as in Aréchiga et al. (1990). In brief, a metal microelectrode of ~10 μm tip diameter was inserted into the cornea while the indifferent electrode (Ag-AgCl) was inserted into the body cavity. Both electrodes were connected to a Dagan EX1 amplifier. The voltage signals were amplified and filtered out for slow movement artifacts. We employed a Grass PS33 photo-stimulator emitting white light pulses of 50 lx for 1 s with intervals of 2 min. This photo-stimulation intensity is within the dynamic range of stimulus–response relationships of these retinal photoreceptors (Picones and Aréchiga, 1990; Aréchiga et al., 1990). The calibration of light intensity was done with a photographic light meter (Goossen, Model Luna-Pro, Germany). Electroretinogram signals were stored in a computer by using the analogical–digital interface micro 1401 coupled to the Spike2 software for analysis (Cambridge Electronic Designed, Cambridge, U.K.). Animals were dark-adapted for 20–30 min. This time is not enough for a full dark adaptation, which takes about 2 to 3 h (Rodríguez-Sosa and Aréchiga, 1982), however our analyses considered this non-stationary condition in the normalization. OA (0.1 ml, 10 μM or 100 μM) and the antagonist phentolamine (100 μM, Gill and Skorupski, 1999) were injected into the hemolymph through a cannula. Substances were obtained from Sigma-Aldrich (St. Louis, MO, USA). The ERG voltage amplitude was determined from the baseline to the peak of the voltage signal. Data were normalized to the *maximum* voltage value in darkness.

In another set of experiments, the ERG was recorded from the isolated retina–*lamina ganglionaris* (R–LG) complex (Rodríguez-Sosa and Aréchiga, 1982), in physiological saline solution slightly modified from van Harreveld (1936) (205 NaCl, 5.4 KCl, 2.6 $MgCl_2$, 13.5 $CaCl_2$, and 10 HEPES, all concentrations in mM, at pH 7.4), by using a suction electrode (~200 μm in diameter tip) loaded with this same saline solution. The electrode was connected to an amplifier (Dagan. Model EX1). The reference electrode (Ag-AgCl) was maintained in the bath fluid. Pulses of white light were delivered as described above. ERG signals were stored and analyzed on a computer as described earlier. The isolated R–LG ($n = 11$) was dark adapted by 20–30 min at 24–26 °C. Then, the test substance was applied to the bathing fluid to reach final concentrations of 1 μM or 10 μM, or 10 mM for phentolamine. These experiments were made between 11:00 and 14:00 h.

2.3. Determination of the retinal pigment position in crayfish

To study the OA effect on both retinal accessory pigments we used 13 animals that were dark-adapted for 2 h between 11:00 and 14:00 h. Five crayfish received an injection of OA (100 μM, 0.1 ml) into the hemolymph under dim red illumination that does not perturb the pigment position. Other eight animals received the same volume of physiological saline solution (slightly modified from van Harreveld, 1936). The animals were then transferred to their containers and kept in darkness for 10 min. The eyestalks were then excised and fixed by heating in boiling water. The position of the pigments was measured by using a micrometer and reported as a proximal-pigment index (PPI) and distal-pigment index (DPI) (Rodríguez-Sosa and Aréchiga, 1982). The PPI and DPI may take values near to zero in the dark-adapted state, or a value of 1 when dispersed in the light-adapted position (Aréchiga and Rodríguez-Sosa, 1997).

2.4. Amine determinations

Determination of OA and Tyr was made by high-performance liquid chromatography (HPLC) following the procedures described by Alvarado-Álvarez et al. (2005). The HPLC procedure and mobile phase were those described by Leung and Tsao (1992), which have been optimized for detection of biogenic amines including OA. The measurements of the content and distribution of OA and its amino acid precursor Tyr were conducted on 25 animals during the photo-phase. The exoskeleton, including muscles and connective tissue sheath was taken out, leaving unharmed the neural part of the optic peduncle. The isolated eyestalks were divided anatomically as follows: retina–*lamina ganglionaris* (R–LG), *medulla externa* (ME), *medulla interna* (MI), *medulla terminalis* (MT) and sinus gland (SG). These structures were excised and stored in ice-cooled saline solution (modified from van Harreveld, 1936). Other groups of five similar structures were pooled and the neural tissue stalks were homogenized in 500 μl of perchloric acid ($HClO_4$; 100 mM) containing 3,4-dihydroxybenzylamine (DHBA) as the internal standard. Samples were then centrifuged at 17,000 RPM by 20 min at 4 °C. The supernatant was removed and filtered through a nylon sieve with a pore diameter of 0.22 μm. Samples were stored at 0–5 °C until their analysis, which was conducted on the same day. The samples were injected into a reverse phase analytical C-18 column (250 mm × 4.6 mm) with a 5 μM particle size (LDC-Analytic). The HPLC included a programmable solvent delivery module (Waters, Model 590), an injector (Model U6K) and a scanning fluorescence detector (Waters, Model 470). The excitation wavelength was 254 nm, and the emission was collected at 338 nm. The data module Waters 730 recorded the signals. The amine solutions were prepared for each experiment and diluted in $HClO_4$ (100 mM). The chromatography procedure was calibrated with the commercial octopamine, L-tyrosine and DHBA (Sigma-Aldrich, St. Louis, MO, USA). The amounts of these substances in the samples were quantified from the peak heights in a linear range of 5 and 35 pmol for OA. The additive range of Tyr was from zero to 2.5 pmol. The flow of mobile phases was set at 0.8 ml/min. The identity of substances was confirmed by increases in the peak amplitude after addition of an external standard. The loss of substances was determined to be 10–15% by using DHBA as the internal standard.

Please cite this article as: Rodríguez-Sosa, L., et al., Octopamine cyclic release and its modulation of visual sensitivity in crayfish, Comp. Biochem. Physiol., A (2016), http://dx.doi.org/10.1016/j.cbpa.2016.08.032