The Effect of Neuropeptides on the ERG of the Crayfish *Orconectes limosus*

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CCAP (Crustacean Cardioactive Peptide), Proctolin, FMRFamide, Met- and Leu-enkephalin, Substance P, RPCH (red pigment concentrating hormone) and PDH (pigment dispersing hormone) were applied to the isolated retina of the crayfish *Orconectes limosus*. Changes in light sensitivity, measured as changes of the amplitude of the electroretinogram (ERG) were observed after application of RPCH, PDH and CCAP. RPCH caused an increase of the ERG amplitude to 133% of its reference value whereas PDH and CCAP decreased the amplitude to 78% and 30% respectively. A dose-response curve showed that 10^{-9} mol/l CCAP produce a half-maximal effect.

Introduction

The chromatophorotropins RPCH (red pigment concentrating hormone) and PDH (pigment dispersing hormone) have been isolated and characterized from the eyestalks of different crustacean species [1–3]. Immunocytochemical studies demonstrated their distribution in the eyestalk and in the central nervous system of decapod crustaceans [4, 5]. PDH and RPCH act primarily on integumental chromatophores but are also known as distal retinal pigment light- and dark-adapting hormones (LAH and DAH) because they mediate the antagonistic movement of distal screening pigments in the retina of decapod crustaceans [6, 7]. In the light-adapted state screening pigment granules are dispersed so that the aperture of the ommatidium for light influx is narrowed in the daytime. Dark adaptation is mainly achieved by distal and proximal concentration of pigment granules resulting in a widened light acceptance angle. These rhythmic changes are accompanied by changes in sensitivity to a constant stimulus measured by the ERG amplitude [8, 9]. The proximal pigment granules within the retinula cells show similar movements which are not regulated by neuropeptides but seem to be controlled by the visual pigment following light absorption [10].

CCAP was originally isolated and characterized from the pericardial organs of the shore crab, *Carcinus maenas* [11]. It is a C-terminally amidated nonapeptid with a disulfide bond. CCAP increases both beat amplitude and frequency of a semi-isolated crab heart. By use of a specific antiserum, CCAP-like immunoreactivity has been found in the eyestalk and the nervous system of the shore crab [12] and three crayfish species [13]. In this study we compare the effects of PDH, RPCH and CCAP on the sensitivity of the isolated crayfish retina with those of other neuropeptides, which have been localized immunocytochemically in the eyestalk of different crustacean species [14–16].

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Materials and Methods

Crayfish, *Orconectes limosus* (Rafinesque), from Berlins river Havel were maintained under constant light conditions (12 h light/12 h dark cycles). Retinas were dissected from compound eyes of the crayfish and mounted in an experimental chamber as described previously [17]. The distal side of the retina was superfused with physiological saline [18] maintained at 15 °C. The flow rate was 1 ml/min. The retina was stimulated every 2.5 min with a flash of white light of 10 ms duration (equivalent photon density $48.75 \times 10^{12}/\text{cm}^2$) and the electroretinogram (ERG) was measured by means of extracellular silver/silver chloride electrodes. The ERG evoked by each stimulus was stored on an IBM-compatible computer. Except for the flashes the retina was kept in total darkness. After a preperiod of 30–40 min the amplitude H_{max} (Fig. 1) of the ERGs reached constant values. Then 10^{-9} to 10^{-6} mol/l of the respective peptide dissolved in an

aliquot of saline were added to the saline superfusing the distal part of the retina. The flow rate was lowered to 0.3 ml/min for the next 20 min allowing the peptide solution a slower passage through the experimental chamber. ERG responses were recorded as described above. In control experiments aliquots of saline containing no peptide were added similarly to the superfusate. Each peptide was tested on 5 crayfish retinas and the percentage of change in the amplitude 30 min after application was determined in respect to the reference value immediately before application of the test substance. All experiments were performed in the early afternoon. Synthetic CCAP, FMRFamide, RPCH, PDH, substance P, Met- and Leu-enkephalin and proctolin were purchased from Peninsula Laboratories.

To establish a dose-response relationship the superfusate contained CCAP in the following concentrations (mol/l): 10^{-8}, 5×10^{-9}, 10^{-9}, 10^{-10} and 10^{-11}. Each concentration was tested on 4–5 retinas as described above.

Results and Discussion

In concentrations of up to 10^{-6} mol/l Substance P, Proctolin, Leu-enkephalin, Met-enkephalin and FMRFamide had no significant effect on the ERG of the crayfish. Under the same conditions CCAP, PDH and RPCH altered the amplitude as shown in Fig. 2 and described below.

Effects of RPCH and PDH

RPCH (DAH) and PDH (LAH) modulate the rhythmic redistribution of retinal pigments [6, 7], which results in circadian variation of retinal sensitivity and can be measured as changes in the ERG [7]. Although these actions have been described previously, we tested the antagonistic effect of the two peptides on the isolated retina for comparative purposes. Application of 5×10^{-9} mol/l RPCH increased the amplitude of the *Orconectes* ERG maximum to $133 \pm 2.3\%$, whereas the same amount of PDH decreased H_{max} to $78 \pm 2.6\%$ of the initial value within the following 30 min.

In organ culture experiments we tested whether the ERG change following the application of PDH was a result of screening pigment migration. Isolated crayfish retinas were incubated at 15 °C in a petri dish containing organ culture medium [19] under a 12 h dark-light cycle (0.18 mW/cm^2). After incubation with PDH during the dark cycle the retinas were fixed. Light microscopic studies of semithin sections showed that the distal screening pigments had moved into the light adapted position

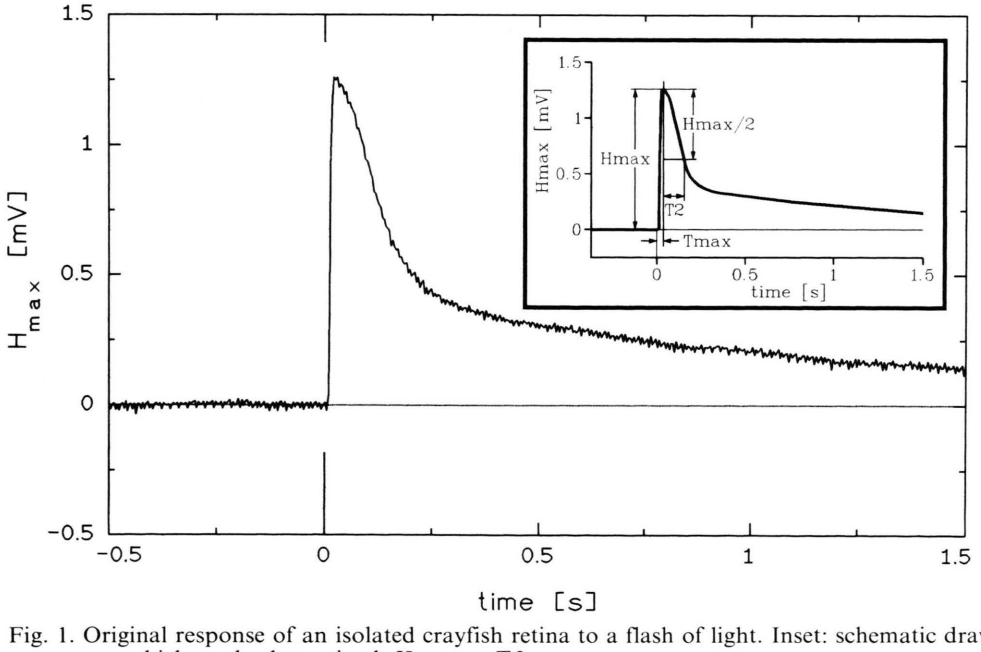

Fig. 1. Original response of an isolated crayfish retina to a flash of light. Inset: schematic drawing of an ERG and parameters which can be determined: H_{max}, t_{max}, T 2.

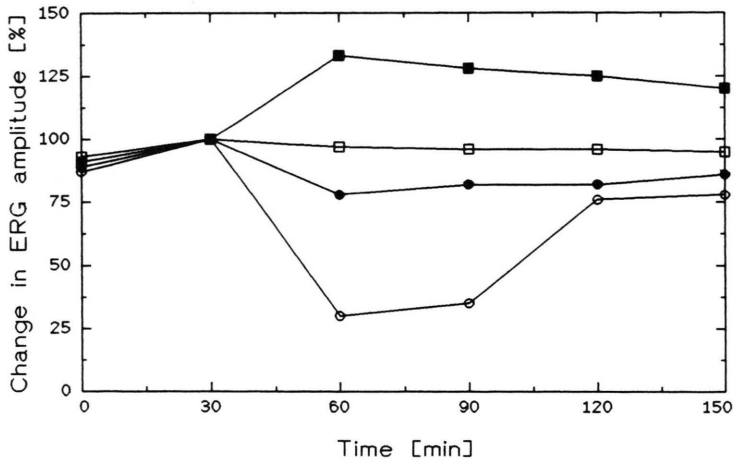

Fig. 2. Effect of CCAP, PDH and RPCH on the amplitude H_{max} of the crayfish ERG. The final concentration of each peptide was 5×10^{-9} mol/l; n = 5, SEM < 3%; filled circles: PDH (LAH); open circles: CCAP; filled squares: RPCH (DAH); open squares: control; begin of application at 30 min.

(Schraermeyer, personal communication). Therefore we suppose that the observed changes in ERG amplitude are brought about primarily by movement of the distal screening pigments.

Circadian ERG changes have been measured in intact eyestalks [20, 21] as well as in isolated eyestalks of the crayfish, *Procambarus bouvieri* [22]. The isolated eyestalk remained in good condition which gave stable recordings up to 72 h. During this time the ERG showed the same general features as in the whole animal including clear circadian ERG fluctuations with differences of more than 200%.

We measured only a total difference of 55% in retinal sensitivity after peptid application. There are some possible reasons for the small responsiveness observed in the isolated retina: firstly and probably mainly it is likely that we destroy some of the distal pigment cells by removing the cornea and crystalline cone. The alignment of the retinulae is destroyed and pigment migration has a smaller effect. Compared to an intact compound eye, light enters the retinula cells of an isolated retina not axially and has to pass pigment-containing tissue. As a result the response to a light flash is generally much smaller. Additionally the humoral control of distal pigment movements by RPCH and PDH might be only part of a complex system of regulation.

Effects of CCAP

The ERG response of the isolated retina to a light flash decreased to $30 \pm 2.9\%$ of the initial value within the next 30 min after application of 5×10^{-9} mol/l CCAP. This effect was reversible, and the amplitude started to increase again 40 min

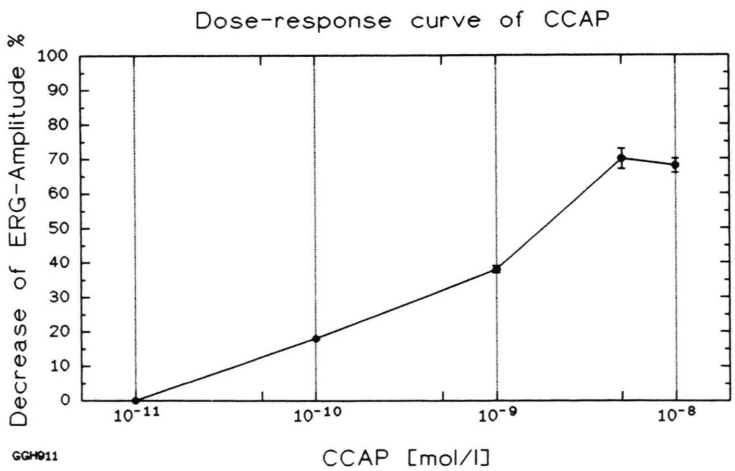

Fig. 3. Dose-response curve of CCAP. The values represent the maximal decrease of the amplitude 30 min after CCAP was added; $n = 4/5$, SEM < 4.5%.

after the CCAP-solution was replaced by saline (Fig. 2). The dose-response relation of CCAP is shown in Fig. 3. Application of CCAP in concentrations above 5×10^{-9} mol/l produced no further decrease of sensitivity. The lowest concentration tested which resulted in a detectable change in sensitivity was 10^{-10} mol/l. This is the first evidence that besides light and PDH also another neuropeptide can regulate retinal sensitivity in crayfish. Proctolin, another crustacean cardioactive peptide, has no detectable effect on the sensitivity of the retina.

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