Octopamine Modulates the Sensitivity of *Limulus* Ventral Photoreceptor

H. Stieve and E. André

Institut für Neurobiologie der Kernforschungsanlage Jülich GmbH, Postfach 19 13, D-5170 Jülich

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The effect of extracellularly applied octopamine (10^{-5} mol/l) on the receptor potential of the *Limulus* ventral photoreceptor was studied.

Octopamine increases the sensitivity of the photoreceptor in the light-adapted state more than in the dark-adapted state. Extracellular application of phentolamine (10^{-5} mol/l) has an opposite, antagonizing effect.

The octopamine effect, though reproducible in the individual experiment, varies greatly from preparation to preparation.

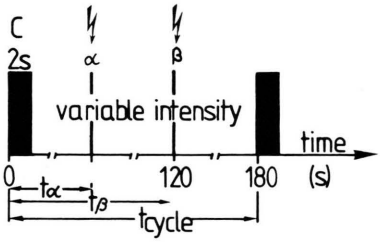
Efferent nerve fibres lead to the rhabdom-containing, light-sensitive part of the ventral nerve photoreceptor cell of *Limulus*, where octopamine, a neurotransmitter substance, is synthesized and released close to the photosensory membrane [1–3]. Kaupp *et al.* [4] found that the level of cyclic adenosine monophosphate (cAMP) in a photoreceptor-rich fraction of a ventral nerve suspension increases after incubation in 10^{-5} mol/l octopamine. This result indicates a physiological role of octopamine via the action of cAMP as a second messenger.

We recorded the receptor potential of the *Limulus* ventral nerve photoreceptor either intracellularly [5] or extracellularly, using the sucrose-vaseline-gap-method [6, 7], and studied the effect of octopamine on the electrical light response. Results have already been briefly reported [8].

The stimulus program consisted in a repeated sequence of three stimuli (Fig. 1 a): A constant conditioning, light adapting stimulus (c) of 2 s duration and high intensity (2×10^{16} photons $\times$ cm^{-2} s^{-1}, 543 $\pm$ 30 nm) was followed by two test flashes.

After a fixed interval t_α following the conditioning stimulus, when the cell is still light-adapted, the first test flash (α, 5 ms) was applied, and 118 seconds after the c-stimulus, when the cell is fairly dark-adapted, the second test flash (β, 5 ms) followed. The interval t_α was adjusted for the individual experiment [5] but varied from experiment to experiment between 8 s and 20 s. The interval t_β was constant for all experiments.

The intensity of the α- and β-test flashes was equal in one sequence and could be varied from sequence to sequence. Our stimulus program by



a

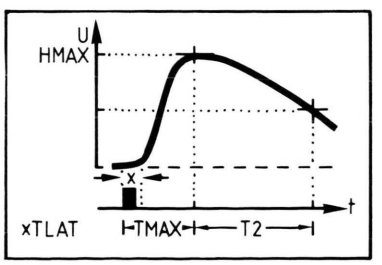


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Fig. 1 a. Stimulus program: C = constant conditioning stimulus, (2 s, 2×10^{16} photons $\times$ cm$^{-2} \times$ s^{-1}, 543 $\pm$ 30 nm). α and β: 5 ms test flashes, intensity varied, maximal intensity: 4×10^{12} photons $\times$ cm$^{-2} \times$ s^{-1}, 543 $\pm$ 30 nm. b. Receptor potential parameters: TLAT = latent period from the beginning of the light-stimulus until the first measurable increase of the response. TMAX = time to peak, between the beginning of the light stimulus and the peak amplitude of the receptor potential. T2 = decrease-time from the peak amplitude (HMAX) to the height HMAX/2.

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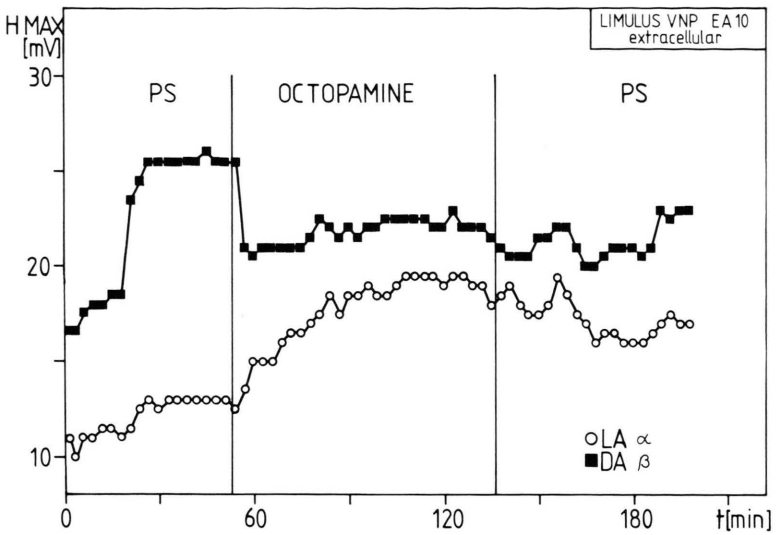
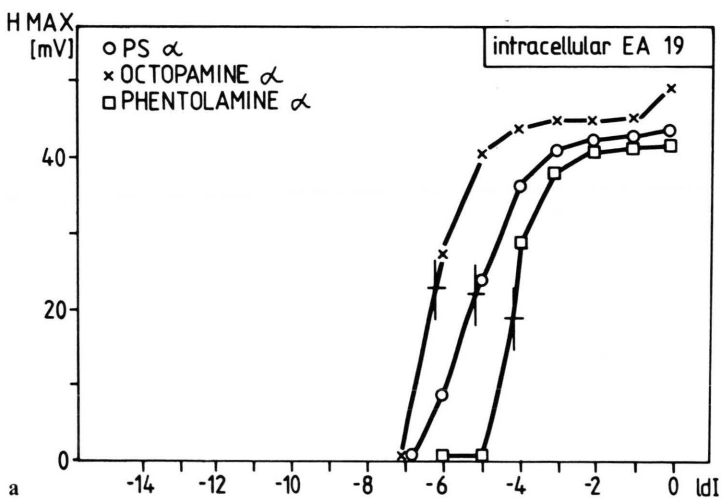


Fig. 2. Effect of extracellularly applied octopamine (10^{-5} mol/l) on the amplitude of the receptor potential (HMAX) of light (α) and dark-adapted (β) photoreceptor cells; $t_\alpha = 11$ s. Extracellular recording. At the beginning of the experiment the light response to the α-flash is half-saturated. It increases after octopamine application to 154% however the light response to the β-flash is reduced to 86%. Further details in Fig. 1.



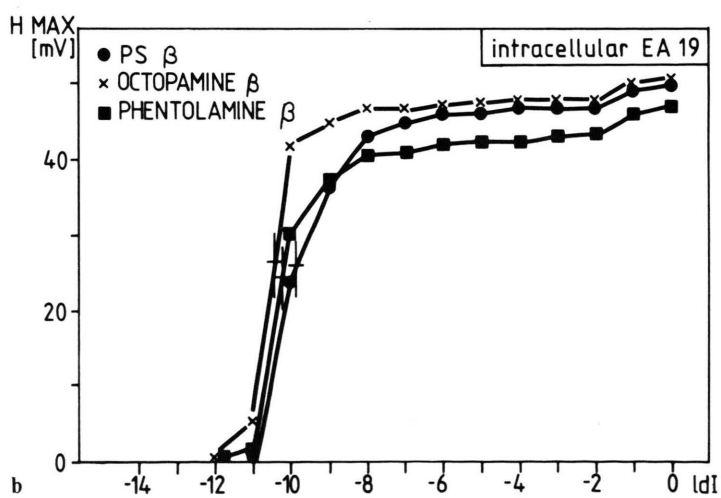


Fig. 3. Effect of extracellularly applied octopamine (10^{-5} mol/l) and phentolamine (10^{-5} mol/l) on the response amplitude versus stimulus intensity curve of a light- and dark-adapted photoreceptor cell. Intracellular recording. $t_\alpha = 17$ s; I_{50} is marked, ld = logarithm to the base 2. Further details in Fig. 1.

which the photoreceptor is reproducibly set into two defined states of adaptation allows us to detect small differences in sensitivity. The shape of the receptor potential was characterized by a number of parameters (Fig. 1 b).

We examined the effect of extracellularly applied octopamine (10^{-5} mol/l) added to the superfusate on the electrical light response of dark- and light-adapted photoreceptors. Phentolamine, which is known to be a blocker of octopamine receptors in several cell types [9], was applied in the same concentration after the exposure to octopamine in order to test the specificity of the observed octopamine effect.

In a group of 3 experiments with extracellular recordings we applied constant test stimuli which evoked a half-saturated amplitude of the α-response. In all 3 experiments the response amplitude of the light-adapted photoreceptor (α) increased under the action of octopamine to about 150%, but that of the dark-adapted photoreceptor (β) was reduced to about 85% (Fig. 2). The octopamine effect became observable as soon as 5 minutes after the beginning of the octopamine application and reached a steady state after about 30 minutes.

In other experiments we reduced the intensity of the test stimuli (α, β) by half from sequence to sequence until electrical light responses could no longer be observed, in order to plot the response amplitude versus stimulus intensity curves of fairly dark- and light-adapted photoreceptors for one group of extracellular and another one of intracellular recordings (Fig. 3, Tables I, II).

The generally weak effect of octopamine was consistent in one experiment, but varied greatly from preparation to preparation. In all experiments the steepness r of the response amplitude *versus* intensity curves (determined at half saturation) as one measure of sensitivity [5], is increased by octopamine (Fig. 3, Tables I, II). In most experiments octopamine shifts the intensity I_{50} of the light stimulus which evokes a half saturated response

Table I. Intracellular recordings. Average values of saturation amplitude HSAT, half saturation intensity I_{50}, steepness r, time parameters TLAT, TMAX, T2 of responses with saturated amplitudes and half saturated amplitudes of light-adapted (α) and fairly dark-adapted (β) photoreceptors employing physiological saline (PS), octopamine containing saline (Oct) and phentolamine constaining saline (Phent). $n = 3$.

	α			β		
	PS	Oct	Phent	PS	Oct	Phent
HSAT (mV)	38.7 ± 1.4	37.3 ± 4.5	39.4 ± 3.7	43.7 ± 6.7	45.1 ± 0.9	44.1 ± 4.0
log I_{50} (I/I_0)	− 1.7 ± 0.1	− 1.7 ± 0.4	− 1.3 ± 0.3	− 2.9 ± 0.3	− 2.7 ± 0.6	− 2.7 ± 0.8
r (mV/decade)	37.4 ± 45.8	55.0 ± 21.9	73.5 ± 23	56.7 ± 33.5	64.2 ± 68.3	48.0 ± 52.8
TLAT (ms) (near HSAT)	30 ± 14	28 ± 8	28 ± 8	30 ± 12	28 ± 7	29 ± 8
TMAX (ms) (near HSAT)	77 ± 10	90 ± 13	71 ± 56	127 ± 56	107 ± 34	109 ± 44
T2 (ms) (near HSAT)	189 ± 48	540 ± 280	834 ± 755	968 ± 417	548 ± 359	2211 ± 500
TLAT (ms) $\left(\text{near}\frac{\text{HSAT}}{2}\right)$	76 ± 47	96 ± 67	39 ± 20	163 ± 81	141 ± 72	164 ± 33
TMAX (ms) $\left(\text{near}\frac{\text{HSAT}}{2}\right)$	142 ± 51	173 ± 91	148 ± 39	453 ± 290	315 ± 227	259 ± 129
T2 (ms) $\left(\text{near}\frac{\text{HSAT}}{2}\right)$	78 ± 18	252 ± 280	255 ± 212	114 ± 26	120 ± 74	203 ± 82

Table II. Extracellular recordings. For further details see legend of Table I.

	α		β	
	PS	Oct	PS	Oct
HSAT (mV)	3.0 ± 0.2	3.4 ± 0.4	3.7 ± 0.5	3.7 ± 0.2
log I_{50} (I/I_0)	−1.54 ± 0.1	−1.36 ± 0.1	−2.2 ± 0.1	−2.1 ± 0.1
r (mV/decade)	0.94 ± 0.6	1.16 ± 0.9	0.78 ± 0.4	0.83 ± 0.4
TLAT (ms) (near HSAT)	18 ± 14	31 ± 10	24 ± 4	27 ± 2
TMAX (ms) (near HSAT)	89 ± 14	114 ± 43	100 ± 30	123 ± 43
T2 (ms) (near HSAT)	50 ± 4	69 ± 19	108 ± 30	152 ± 33
TLAT (ms) (near I_{50})	52 ± 14	52 ± 15	81 ± 1	76 ± 22
TMAX (ms) (near I_{50})	128 ± 9	137 ± 17	217 ± 47	284 ± 27
T2 (ms) (near I_{50})	67 ± 17	83 ± 17	100 ± 29	125 ± 38

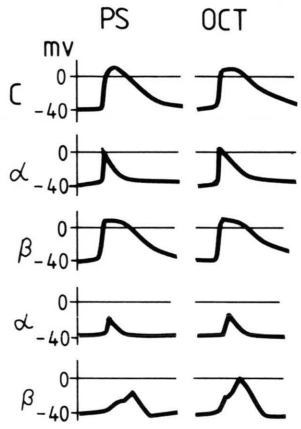


Fig. 4. Intracellularly recorded receptor potentials in physiological saline, PS, (left side), and after application of 10^{-5} mol/l octopamine (right side). First line: responses to conditioning illumination (c); second and third line: α and β responses, ld $I/I_0 = 1$, fourth and fifth line: α and β responses, close to half saturation, (α response, ld $I/I_0 = 6$, β response, ld $I/I_0 = 10$). Further details in Fig. 1. Same experiment as in Fig. 3.

amplitude (another measure of sensitivity) to higher light intensities (Fig. 3, Tables I, II). This effect is often larger in the light-adapted than in the dark-adapted photoreceptor. After application of octopamine the saturated amplitude (HSAT) of the receptor potential of photoreceptor cells in both states of adaptation is somewhat increased (Fig. 3). No influence on the prestimulus membrane potential (PMP) was observed.

In these experiments we can find an octopamine effect on both light- and dark-adapted cells, but more marked for the light-adapted cells (Fig. 3). The shape of the receptor potential is not much changed under the influence of octopamine (Fig. 4, Tables I, II). On the average the effects on the extracellular recorded receptor potentials are more pronounced than those on the intracellular recorded ones. The time course of the receptor potential is slowed down especially the decline of the receptor potential, characterized by the decrease time T2 (Tables I, II).

Addition of phentolamine (10^{-5} mol/l) to the physiological saline after octopamine application causes a decrease of the saturated amplitude of the receptor potential (Fig. 3) and a sensitivity increase, characterized by a shift of I_{50} to lower intensities. However the steepness r of the response amplitude versus intensity curve is not reversed by phentolamine (Fig. 3, Table I) and phentolamine does not antagonize the octopamine effect on the shape of the receptor potential.

Our results – in spite of the great variability of the octopamine effect – support the idea that the sensitivity of the ventral photoreceptor is weakly modulated by efferent octopaminergic nerve fibres. Octopamine seems to have an influence on the adaptation process because the cells dark-adapt faster after octopamine application [10, 11]. The fact that the sensitivity of the light-adapted photorecep-

tor is more increased by octopamine than that of the dark-adapted is explained by this observation.

O'Day and Lisman [10, 11] studied the octopamine effect on the ventral photoreceptor of *Limulus* by the use of the voltage clamp technique parallel to us. They show that octopamine enhances dark-adaptation.

The changes in the shape of the receptor potential, especially the retardation of the decline expressed by the increase of T2, could favour the assumption that octopamine acts on the intracellular level of calcium ions, which is also proposed by O'Day and Lisman [10, 11]. However the finding that phentolamine does not antagonize the octopamine effect on the shape of the receptor potential does not support this hypothesis.

Perhaps the most prominent observation is the great variability of the octopamine effect among different preparations. This may have something to do with the physiological conditions of the preparation. The effect of octopamine may depend on a diurnal rhythm and therefore the time when the animals were killed could be important. In 7 experiments in which Limuli were killed at eight o'clock in the morning we could observe an influence of octopamine on the amplitude of the receptor potential.

On the other hand, 5 experiments in which the animals were sacrificed in the afternoon showed no octopamine effect on the amplitude of the receptor potential. However, an influence on I_{50} or the steepness of the curve was sometimes observed.

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