Octopamine levels during the moult cycle and adult development in the migratory locust, *Locusta migratoria*

Suzel Fuzeau-Braesch, J.F. Coulon[2] and J.C. David

Laboratoire de Biologie de l'Insecte, Bat 440–443, Université de Paris-Sud, F-91405 Orsay (France), U.E.R. de Biochimie, Université d'Angers, bd Lavoisier, F-49 Angers (France), and Laboratoire de Biochimie du Développement, I.R.B.M. Université Paris 7, 2, place Jussieu, Paris 5 (France), 23 October 1978

Summary. Octopamine content of the head of the locust *Locusta migratoria* has been determined during the last larval stage, moulting and adult life of 3 groups of insects: female and male gregarious, solitary and CO_2 solitarized. An important difference was found between these 3 groups. Octopamine contents increased in the middle of the larval life and during the adult life. The moulting time is characterized by a sharp decrease of the octopamine content which becomes identical in the 3 groups of insects. The relation between octopamine content, hormone cycles and motility is discussed.

It has recently been shown that octopamine contents of the head of the migratory locust *Locusta migratoria*[1] varies as a function of the phase state of the animal[2]. We found a 2–2.5-fold increase when the animals are solitary or artificially solitarized by a CO_2 treatment in adult mature males, as compared to gregarious insects. Another change was shown for dopamine, where an increase in gregarious insects as compared with solitary[3] is seen.

To understand the significance of this conspicuous change in octopamine content, found in mature males, we have now measured the content of the amine at different ages: during the last larval stage at 2-3, 4-5, 6-7 days, 1 day before ecdysis, just at the ecdysis, and in the adult, young 1 day and 5 days after ecdysis, and mature (15 days for solitary and CO_2 treated, 21 days for gregarious because of the phase influence to the sexual evolution), for both sexes. Solitary locusts are obtained by isolation at emergence; the gregarious group consist of 200 insects crowded in one cage of $40 \times 40 \times 40$ cm. CO_2-treated animals are solitarized by a chronic treatment of 1 min per day during all the larval life time[4]. All physical conditions are identical (photo- and thermoperiod of 12/12 h, 25–35 °C) and experiments simultaneously performed for the 3 groups of animals. Strain used is *cinerascens* from Sardinia.

Octopamine is measured after Molinoff and Axelrod method[5]. Heads of locusts are individually mixed with a Polytron in 10 vol. of cold Tris-HCl 0.05 M (pH 8.6) with pargyline (1 mM). Extracts are centrifuged for 5 min at $20{,}000 \times g$ and the supernatants heated for 3 min at 95 °C and then centrifuged again. After a 1:10 dilution, 150 µl of the supernatants are incubated at 37 °C with 37.5 µl of PNMT (phenylethanolamine-N-methyl transferase) and 0.04 nmoles of [^{3}H] SAM ([^{3}]methyl-S-adenosyl-l-methionine from C.E.A. Saclay, 13.5 Ci/nmole) in 60 µl phosphate buffer 50 mM, pH 8.6. The reaction was stopped after 45 min with 200 µl of borate buffer 0.5 M (pH 11) sodium chloride saturated, with 1 µg of p.synephrine. Then, extraction is made with 2.5 ml of ethylacetate and a 0.5-ml aliquot is completly evaporated. Residual product is dissolved in 1 ml absolute ethanol and the radioactivity counted with toluene PPO-POPOP as scintillation liquid. Authenticity of octopamine was checked using the dansyl derivative[6] and the 2 consecutive TLC separations: chloroform-N-butylacetate 5:2 (v/v), Toluene-triethylamine-methanol 50:5:1 (v/v). The R_f-values for the dansylated methylated amines in these 2 systems were 0.36 and 0.34, respectively, thus positively identifying the amine as octopamine.

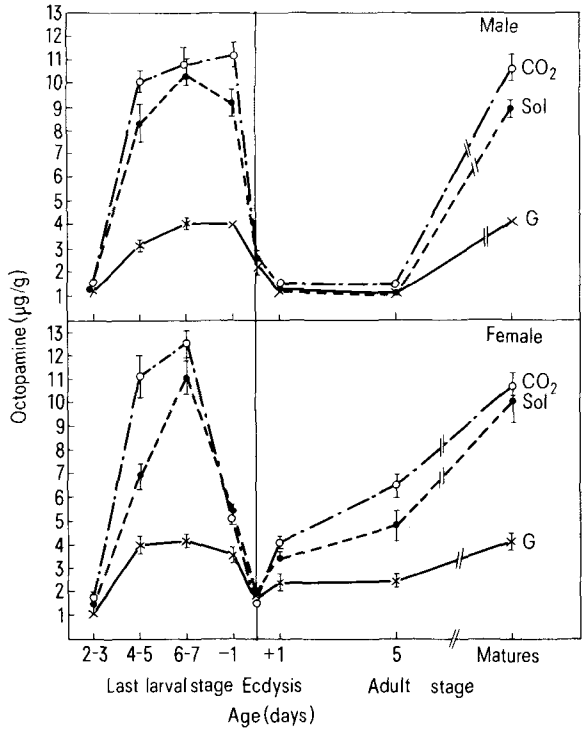
Results are presented in the figure. A moult cycle of large amplitude is found in both sexes for the 3 groups of insects in the content of octopamine. The highest values are obtained in the middle of the larval stage and in the mature animals. Ecdysis for females, ecdysis and following days for males give the smallest values. At their highest values, the 3 groups of insects are strongly differentiated: CO_2-treated and solitary are very rich in octopamine: 8–12 µg/g, as compared with gregarious: 3–4 µg/g. On the contrary, the moulting time is characterized by a sharp decrease of the octopamine content which becomes identical in the 3 groups of insects.

Considering the known hormonal development during the same period, it is evident that the observed changes in octopamine content can be directly related neither to the moult hormone (ecdysone), nor to the juvenile hormone or to the bursicon:

a) Maximum activity of ecdysone is found at the 6th day of the larval cycle followed by a definitive low level in the imaginal state[7,8].

b) Juvenile hormone decreases slowly during all the larval state and presents a sharp peak mainly in solitary insects, at the ecdysis. Then the activity shows a progressive increase during the sexual maturation[7-9].

c) Bursicon presents a sharp single peak of activity after the ecdysis[10], related only with sclerotization and darkening of the cuticle[11].



Octopamine levels related to age and way of life (phase) in *Locusta migratoria*, in µg/g: last larval stage and adult. Ages in days; −1 and +1 = 1 day before and after ecdysis. Matures: 15 days for solitary and CO_2 treated, 21 days for gregarious insects. G = gregarious, Sol: solitary, CO_2 = CO_2 solitarized animals. Each value is the mean ± SEM of 3 animals except for adults: 4–7 animals.

On the contrary, the cyclic differences may be exactly related to the motility of the animals which have been previously described, as similar evolutions, in our laboratory with the same strain[12]. This observation may support the assumption of Evans[13] that octopamine may play a similar role as compared to its catechol analogue noradrenaline in the vertebrate nervous system. But, except at the ecdysis, a great difference appears between gregarious and both solitary groups where octopamine contents are always at the highest level. Since gregarious animals are much more active than solitaries[12] and show the smallest values of octopamine, it becomes contradictory to claim that motility is directly related to the contents of octopamine. It leads us to consider that the genuine situation must be related to the pool of amines, particularly dopamine[3] and thus gives rise to the problem of the differentiation of the specific receptors in both types of animals, gregarious and solitary. In fact, we recently showed that specific receptors exist for octopamine in the locust brain[14]. So the phase differentiation may correspond to a differential organization of receptors during the ontogenesis since octopamine is now well-recognized as a neurotransmitter[15-18].

1 S. Fuzeau-Braesch and J.C. David, C.r. Acad. Sci. D, Paris *286,* 697 (1978).
2 B. Uvarov, *Grasshoppers and Locusts,* vol. 1. Cambridge University Press, G.B. 1966.
3 S. Fuzeau-Braesch, C.r. Acad. Sci. D, Paris *284,* 1361 (1977).
4 G. Nicolas, Acrida *1,* 97 (1972).
5 P.B. Molinoff and J. Axelrod, Science *164,* 428 (1965).
6 J.C. David, Ann. Biol. Biochem. Biophys. *17,* 1101 (1977).
7 L. Joly, J. Hoffmann and P. Joly, Acrida *6,* 33 (1977).
8 P. Porcheron, P. Cassier and F. Dray, Bull. Soc. zool. Fr., suppl. 1, 71 (1977).
9 R.A. Johnson and L. Hill, J. Insect Physiol. *19,* 1921 (1973).
10 G. Fraenkel and C. Hsiao, J. Insect Physiol. *11,* 513 (1965).
11 J.F.V. Vincent, J. Insect Physiol. *17,* 625 (1971).
12 G. Nicolas, Rev. comp. Anim. *6,* 255 (1972).
13 P.D. Evans, J. Neurochem. *30,* 1009 (1978).
14 J.C. David and S. Fuzeau-Braesch, C.r. Acad. Sci., Paris *288,* 1207 (1979).
15 H.A. Robertson and J.E. Steele, J. Physiol. *237,* 34 (1973).
16 H.A. Robertson and A.V. Juono, Int. Rev. Neurobiol. *19,* 173 (1976).
17 H.A. Robertson, Experientia *32,* 552 (1976).
18 P.D. Evans and M. O'Shea, Nature *270,* 257 (1977).

Effect of adapting target size on the gain of the surround response mechanism in X- and Y-cells in cat retina

W.G. Christen, R.W. Winters, H.I. Cohen and T.W. Robertson

Department of Psychology, University of Miami, Box 248185, Coral Gables (Florida 33124, USA), and William L. McNight Vision Research Center, University of Miami School of Medicine, 1638 N.W. 10th Avenue, Miami (Florida 33152, USA), 28 December 1978

Summary. The adaptation field of the surround mechanism of X and Y retinal ganglion cells in the cat was assessed with variable size, unmodulated adapting spots. Both an on-inhibition measure and an off-discharge measure of surround gain was used. Results suggest that the surround mechanism in Y-cells is strongest in the receptive field middle but weak or nonexistent in the middle of X-cell receptive fields.

The activity of cat retinal ganglion cells is thought to be controlled by 2 processes which overlap spatially[2]. These processes are referred to as the center and surround response mechanisms and initially it was believed that each mechanism could be described by a Gaussian curve which peaked in the middle of the receptive field. Studies over the past 13 years indicate that retinal ganglion cells can be divided into at least 2 subgroups based on their responses to a number of different stimuli[3-11], and are most commonly reffered to as Y- and Y-cells[3]. Recent investigators[12,13] have suggested a difference in the spatial distribution of the surround mechanism in X- and Y-cells; they postulate that the surround mechanism is most sensitive in the middle of the receptive field of Y-cells but weak or nonexistent in the middle of X-cell receptive fields.

One way to assess the spatial distribution of the surround mechanism is to determine the mechanism's adaptation receptive field. This method assumes that the adaptation receptive field for the surround mechanism corresponds to its signal receptive field. Several studies have shown this to be the case for the center mechanism[7,14,15]. In the experiments to be described here we assessed the adaptation field of the surround of X- and Y-cells with variable size, unmodulated adapting spots.

Material and methods. Lacquer coated tungsten microelectrodes were used to record the action potentials of 41 on-center optic tract fibres from lightly anesthetized (Nembutal) adult cats. Details of the animal preparation, recording system and optical system are described elsewhere[16]. A contrast reversal stimulus[3,17] was used to classify the cells as X or Y: this stimulus consisted of a bipartite spot (3.0°) centered in the receptive field. The light and dark hemifield was reversed (0.5 Hz) by cross polarizing filters. At the null position the response of X-cells to the light and dark reversal of the target was unmodulated. Y-cells, however, responded with 'on transients' at each reversal of the hemifield. All flashing stimuli in the study were rectangular in time with a duration of 500 msec and frequency of 0.3 Hz and were superimposed upon a steady background of 3.1 candles/m^2. Receptive field centers were typically 0.7–1.2° for X-cells and 1.5–2.0° for Y-cells.

2 experiments were conducted. In the first experiment the effect of variable size adapting spots (the luminance of which was adjusted to keep the gain of the center mechanism constant) upon the gain (defined as the ratio of response magnitude to stimulus magnitude) of the surround mechanism was measured. The gain of the surround mechanism was assessed by measuring the ability of a flashing annulus ($4.0° \times 10.5°$) in the periphery of the receptive field to suppress an excitatory response generated by a flashing center spot placed in the middle of the receptive field. In the second experiment variable size, equal flux (i.e. luminance x-area was held constant) spots were used as the adapting targets. The measure of surround gain was the off-discharge produced by a flashing annulus ($4.0° \times 10.5°$) placed in the receptive field periphery.

Results and discussion. Results from a typical X- and Y-cell in the first experiment are shown in figure 1. On the abscissa is plotted adapting spot size and on the ordinate is percent inhibition. Percent inhibition is defined as the ratio