Octopaminergic control of corpora allata activity in an insect

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Summary. Octopamine enhanced the release of JH3 from isolated corpora allata of locusts in short-term cultures. This effect was suppressed when phentolamine (inhibitor of the octopamine receptors) was added to the culture medium. Moreover an octopamine-sensitive adenylate cyclase was found in the corpora allata. The results suggest a positive octopaminergic control on the activity of the corpora allata.
Key words. Octopamine; juvenile hormone; corpora allata; locust.

The presence of octopamine in the corpora allata (CA) of the locusts, *Schistocerca americana*[13] and *Locusta migratoria*[12], suggests that this amine plays a role in the control of the juvenile hormone (JH) secreting cells. This paper presents support for the role of octopamine in JH release by the isolated CA from adults of *Locusta migratoria migratorioides*.

Materials and methods. Thirty-day-(±5)-old adult males of *Locusta migratoria migratorioides* reared in gregarious conditions[8] were used. The production of juvenile hormone (JH3) by an individual isolated CA is constant for more than 24 h, and is equivalent to the hormone synthesized, because no storage occurs in the gland[3,7]. Even though animals of the same physiological age were used, and even when the right and left glands of the same animal were compared[3,29], the production by different CA was very variable, from 5 to 50 ng/ml/h. In order to obtain comparable data, the following procedure was applied. Each CA was successively incubated for 12 h (adaptation period), then 2 h in the culture medium alone (basal period = B), and finally incubated for 2 h in the culture medium containing the substances to be tested (experimental period = E). For each CA, the concentration of JH3 measured during the experimental period was expressed as a percentage of that measured during the basal period, i.e. 100(E-B)/B; under all the experimental conditions used the mean was calculated from at least 20 different assays. Sterile techniques were used throughout all phases of the study.

Incubations were performed at 34 °C (±1 °C) with constant gentle shaking in silicone-coated glass tubes containing 1 ml of the S20 culture medium[3,7,24] with 2% of fetal calf serum only (controls, C), or containing 10^{-4} M octopamine (DL-p-octopamine hydrochloride, Sigma: OA), or 10^{-4} M phentolamine (Regitine, Ciba-Geigy: PH), or both substances together (OA + PH). No other molarities were tested, on two grounds. Firstly, a 10^{-4} M octopamine concentration had a just significant action (see results) in comparison with the strong variations of the individual reactions, therefore lower molarities would not have supplied any further information. Secondly, the concentration of 10^{-4} M corresponds, for octopamine, to the concentration of maximum known efficiency[14], therefore there is no value in testing greater concentrations. After incubation the tubes were frozen and kept at −30 °C until used for radioimmunoassays (RIA); known quantities of JH standard kept under these conditions were not modified for many weeks.

The production of JH1, 2 and 3 was analyzed by RIA according to the method previously described[2,3]; only JH3 was detected, as observed in previous work[4,10,11]. The RIA was carried out in duplicate directly in 100 µl of the culture medium and the standard curve was prepared with the culture medium.

The adenylate cyclase activity was determined as previously described[5,23]. The CA were isolated and transferred to ice-cold 2 mM tris maleate buffer, pH 7.2, containing 2 mM EGTA (10–15 pCA/100 µl buffer). The tissues were homogenized at 0 °C, filtered through a nylon screen of 100 µm pore diameter and used as the source of adenylate cyclase. The incubation was initiated by adding the CA homogenates containing 10–20 µg of proteins and lasted 10 min at 30 °C. Under the experimental conditions used, cyclic AMP formation was linear for incubation times of up to 15 min. Each experimental point was assayed in triplicate, the variation of the data did not exceed 5%. Adenylate cyclase activity was expressed as pmol cyclic AMP formed per min per mg of protein in the homogenate. The protein level was determined by colorimetry[24] using bovine serum albumine as standard (fraction V, Sigma).

Results. The production rate of JH3 of the controls (C, fig. 1) exhibited a significant increase (+146%, $p < 0.001$) when the CA were transferred into fresh medium, i.e. from the basal to the experimental period. When the CA were transferred into fresh medium containing the mixture of octopamine and phentolamine (OA + PH), the production rate of JH3 was unchanged compared with that of the controls (C). When the CA were transferred into a standard medium containing octopamine (OA), JH3 synthesis was stimulated to give 4 times the basal production ($p < 0.001$). This was more than twice the increase measured when the controls were transferred into a medium without octopamine. When the CA were transferred into a standard medium containing phentolamine (PH), the JH3 synthesis was not different from that of the basal period ($p = 0.5$). In other words, the phentolamine suppressed the stimulating effect induced by the transfer of the glands into a fresh medium.

Figure 2 shows the dose-response of the stimulation of CA adenylate cyclase by octopamine. A maximum stimulation of about 1.2 times the basal activity was reached with 3.3×10^{-5} M octopamine. The K_A app (concentration of agonist giving 50% of maximal stimulation) was 3 µM.

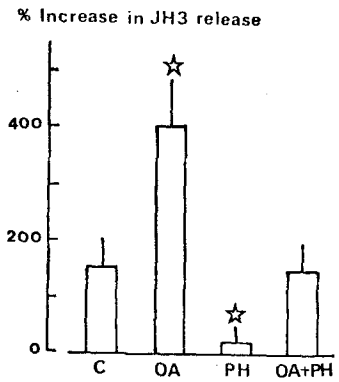

Figure 1. Effects of octopamine and phentolamine on the release of JH3 by corpora allata in vitro. Results are means ±SEM of the increase in JH release during the experimental period (incubation in a medium without (control, C) or with octopamine (OA), phentolamine (PH), or octopamine plus phentolamine (OA + PH), as a percentage of JH release during the basal period (incubation in control medium). Star: $0.01 < p < 0.02$ compared to controls.

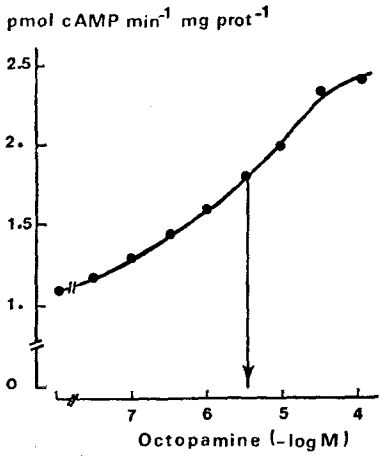

Figure 2. Stimulation of corpora allata adenylate cyclase by octopamine. Values are averages of triplicate measurements from a representative experiment. The variation was less than 5%. Vertical line indicates the K_A app.

Discussion and conclusions. The increase of JH release in response to the transfer of the CA was enhanced by the presence of octopamine in the medium and this effect was suppressed when phentolamine (a good inhibitor of the three octopaminergic receptors known in the locust [14]) was added to octopamine. These data indicate that the potentiation of the JH release is mediated at least through octopamine-receptors. Moreover, the stimulation of the CA adenylate cyclase by octopamine confirms the presence in the corpora allata of octopaminergic receptors coupled with an adenylate cyclase. We know that inside each CA the JH-synthesizing cells adjoin different types of nervous axons originating in the brain [9, 16, 20], and perhaps also in the subesophageal ganglion through the esophageal nervi. Some of these axons are peptidergic [18, 19, 26] and others could be octopaminergic since, in extracts of CA, octopamine was detected by radioenzymatic assay [12, 13]. Moreover, because octopamine is a neurohormone in insects [27], the diffusion of octopamine into the glands from the hemolymph may be taken into account, as recently suggested for corpora cardiaca [21].

The present results offer evidence for two possible mechanisms. First, octopaminergic receptors could be located in the membrane of the peptidergic varicosities; octopamine would then stimulate the allata cells via the peptidergic axons and act on the release of JH as a neuromodulator. Alternatively, in the other possible mechanism, octopaminergic receptors would be present in the membranes of the allata cells and octopamine would then directly control the release of JH.

JH release was strongly inhibited by addition of phentolamine to the standard medium, compared to that for the controls. The stimulation which was generally observed after the transfer of CA in the control medium disappeared. Thus the inhibition of octopamine receptors suppressed the activation of the CA due to the transfer. This result suggests a continuous stimulation of allata cells by octopamine in short-term cultures: during the first days of the culture the sectioned octopaminergic endings present in the CA may release octopamine before degenerating (which usually follows the separation from the originating cells [17].

As far as we know, only neuropeptides of cerebral origin have been tested as regulators of the CA activity in insects [18, 19, 26]. Our results confirm the complexity of the neuronal patterns involved in the control of insect endocrine glands [21, 29, 30] and emphasize the importance of the role of octopamine, which is so widely distributed in insect tissues [27].

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