THE STIMULATION OF DOPA DECARBOXYLASE ACTIVITY BY ECDYSONE AND ITS ENHANCEMENT BY CYCLIC AMP IN ADULT MOSQUITOES*

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SUMMARY: Adult Aedes aegypti mosquitoes exhibit a low level of dopa decarboxylase activity. When non-blood fed females are injected with the molting hormone β-ecdysone a considerable increase in the level of enzymatic activity is observable. This β-ecdysone mediated stimulation is significantly enhanced when the hormone and dibutyryl cyclic AMP are injected simultaneously. No significant increase in dopa decarboxylase activity is detectable when dibutyryl cyclic AMP is injected alone.

Very little dopa decarboxylase activity is detectable in adult female Aedes aegypti prior to blood feeding. However, once fed, the enzymatic activity, after a 24 hour lag rises dramatically and continues to increase until approximately 72 hours, whereupon it levels off. The ingestion of a blood meal in these anautogenous females is necessary for oocyte maturation, and normal mature eggs contain essentially all of the dopa decarboxylase activity found in these females. Newly oviposited eggs darken and harden through a process much like sclerotization, thus accounting for the presence of dopa decarboxylase (1).

In order for sclerotization of dipteran puparia to occur, the presence of the sclerotizing agent, N-acetyldopamine is necessary. This compound is derived from tyrosine which is hydroxylated to form 3,4-dihydroxyphenylalanine (dopa), the dopa is then decarboxylated to produce 3,4-dihydroxyphenylethylamine (dopamine) and finally, acetylation of dopamine leads to N-acetyldopamine. Additional studies then centered on the decarboxylation step, and it was con-

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cluded that the enzyme, dopa decarboxylase (E.C.4.1.1.26) is induced by the molting hormone, ecdysone (2).

Since dopa decarboxylase is induced by ecdysone in the pre-adult stages of other insects, it is logical to ask whether or not the hormone will increase dopa decarboxylase activity in non-blood fed, adult, Aedes aegypti females. We report here that the answer to this question is affirmative, and we will discuss the important implications of this observation with regard to the relationship of ecdysone and ovarian development. Moreover, we have also found that dibutyryl cyclic AMP will, in the presence of β-ecdysone further increase the enzymatic activity, but the nucleotide by itself elicits no stimulation whatsoever.

MATERIALS AND METHODS

Dopa decarboxylase activity, was obtained by measuring the conversion of radioactive substrate, [2-^{14}C]DL-Dopa, to product, [1-^{14}C]Dopamine. For each assay, 40 to 50 mosquitoes were homogenized in 8 volumes (w/v) of 0.04 M sodium phosphate, pH 7.2, buffer containing 0.2 mg/ml 1-phenyl-2-thiourea (PTU). The homogenates were centrifuged at 41,000 x g for 30 minutes at 0°C and the resulting supernatants were filtered through glass wool and then incubated with substrate and pyridoxal-5'-phosphate for 10 minutes at 37°C. Separation of product and substrate and subsequent monitoring of radioactivity was accomplished essentially using the radiochromatographic procedure of Lunan and Mitchell (3). Protein concentration was determined by the method of Lowry et al. (4).

Adult mosquitoes were injected intrathoracically. The injection apparatus consisted of a mouthpiece, rubber tubing, a Leitz micromanipulator needle-holder, and an injection needle made by drawing a capillary tube to a fine point. Routine injections involved a sample dose of 1.6 ± 0.1 μl.

β-ecdysone was obtained from Rohoto Pharmaceutical Co., Osaka, Japan and N^6,O^2-dibutyryl adenosine 3':5'-cyclic monophosphoric acid (hereafter referred to as cyclic AMP) from Sigma. The solvent in all cases was Aedes

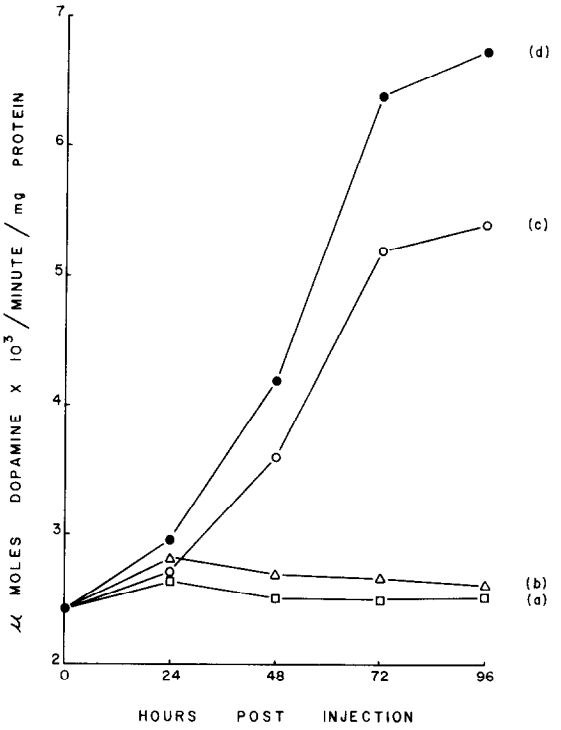

Figure 1. Effect of injecting β-ecdysone and dibutyryl cyclic AMP on DOPA decarboxylase activity in adult, non-blood fed Aedes aegypti females: Curve a, saline; Curve b, cyclic AMP (5.0 μg/μl); Curve c, β-ecdysone (5.0 μg/μl); Curve d, β-ecdysone + cyclic AMP (5.0 μg/μl of each). The volume injected in all cases was 1.6 μl ± 0.1 using Aedes physiological saline as a solvent. Each point represents the average value of at least 3 replicates. The maximum range of variability between replicates was less than 10% in all cases.

physiological saline (5). The rearing procedures for the mosquitoes have already been described (6).

RESULTS

It is clear from Figure 1 (curve c) that non-blood fed females who have received an injection of β-ecdysone showed a marked increase in dopa decar-

boxylase activity compared to the saline injected controls (curve a). Injection of mouse blood, mouse serum, cholesterol and a juvenile hormone mimic (Williams-Law mixture) did not by themselves result in any increase in enzyme levels whatsoever. Interestingly, no increase in enzymatic activity was observable in adult males who were injected with ecdysone as well.

Ecdysone is known to induce specific polytenic chromosomal puffs, which are thought to be the direct morphological expression of gene activity (7). Using isolated Drosophila hydei salivary glands, Leenders et al (8) showed that cyclic AMP will enhance β-ecdysone induced puffs but that cyclic AMP alone did not. Since ecdysone is thought to induce dopa decarboxylase in pupating Calliphora, we then asked what would the effect of cyclic AMP be on dopa decarboxylase activity in adult Aedes females. Accordingly then, one group of non-blood fed females was injected with β-ecdysone alone, another with cyclic AMP alone, and a third group with β-ecdysone and cyclic AMP simultaneously, and their dopa decarboxylase activities, post-injection, were determined. It is apparent from Figure 1, that administration of cyclic AMP by itself (curve b) does not result in a significant increase in enzymatic activity compared to the saline injected controls (curve a). However, Figure 1 does show that the simultaneous injection of β-ecdysone and cyclic AMP (curve d) does significantly increase the dopa decarboxylase activity above that of individuals injected with β-ecdysone alone. The magnitude of the synergistic effect of cyclic AMP in the presence of ecdysone is best seen by referring to Table 1, whereby it is shown that by 72 and 96 hours respectively, approximately 50% more enzymatic activity is detectable compared to that obtained with β-ecdysone alone.

DISCUSSION

For the first time it has been demonstrated that β-ecdysone will stimulate the activity of a particular enzyme in an adult insect. Secondly, cyclic AMP will enhance this effect only in the presence of the hormone, but will not by itself elicit any significant increase in dopa decarboxylase activity.

TABLE 1. The Enhancement of Ecdysone Mediated Dopa Decarboxylase Activity by Dibutyryl Cyclic AMP in Adult non-blood fed Aedes aegypti females.

Hours after injection	% Increase of Dopa Decarboxylase Activity Compared to Saline Injected Control: Cyclic AMP injected alone	β-ecdysone injected alone	Cyclic AMP + β-ecdysone injected simultaneously	% stimulation* by Cyclic AMP in presence of β-ecdysone
48	7	43	68	18
72	6	108	157	43
96	2	117	170	51

* This value represents the net percent increase. It was calculated as follows:

(Cyclic AMP + β-ecdysone) - [(Cyclic AMP alone) + (β-ecdysone alone)].

From this we conclude that in this system, cyclic AMP is not acting as a second messenger for ecdysone. The slight increase in enzymatic activity seen at 24 hours in all cases is probably a reflection of the normal wound response found in many insects (9).

The role of ecdysone in normal ovarian development in Aedes aegypti is unclear. Spielman et al (10) found that exogenously administered β-ecdysone will trigger ovarian development in non-blood fed A. aegypti, and Fallon and Hagedorn (11) state that injection of ecdysone into unfed females activates vitellogenin synthesis by the fat body. Thus, it seems likely that the increased dopa decarboxylase activity we see after injection of β-ecdysone is a small part of the overall metabolism associated with oocyte maturation. Since of the substances tested only β-ecdysone results in an increase in enzymatic activity, and only in females, specificity is suggested. This leads us to suggest that the blood meal, normally needed for ovarian development

in Aedes aegypti somehow triggers the endogenous release, activation or synthesis of this hormone in adult females. We are in the process of testing this hypothesis by directly assaying for ecdysone levels of females before and after a blood meal.

A second messenger role for cyclic AMP in insects has been indicated in other insect systems. Berridge (12) and Maddrell et al (13) found that serotonin induces fluid secretion in isolated Calliphora salivary glands and isolated Malpighian tubules of Rhodnius and Carausius. Treatment of these isolated organs with cyclic AMP alone produced the same effects. However, the interaction of cyclic AMP and ecdysone is nebulous. Rojakovick and March (14) report that β-ecdysone inhibits cockroach brain adenyl cyclase, while on the other hand Applebaum and Gilbert (15) found that the cyclase activity in Hyalophora gloveri pupal wing epidermis is stimulated by the hormone. Our work reported herein, indicates that cyclic AMP is not a second messenger for ecdysone but rather it enhances the ecdysone effect. This exactly parallels the cyclic AMP effect seen in ecdysone mediated chromosomal puffs, referred to previously. Obviously numerous intriguing speculations are possible to explain these observations, but however, we will defer from doing so until more extensive data is in hand.

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