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Ovarian ecdysone secretion is controlled by a brain hormone in an adult mosquito

H. H. Hagedorn, J. P. Shapiro & K. Hanaoka

Department of Entomology, Cornell University, Ithaca, New York 14853

In the mosquito *Aedes aegypti*, a blood meal triggers egg development. Early workers used the simple technique of decapitation, or neck ligation, to show that a factor from the head was required for normal egg development[1-4]. Lea subsequently demonstrated that the source of this factor was the medial neurosecretory cells of the brain[5]. The hormone from these cells, which he termed the egg development neurosecretory hormone (EDNH), is stored in the corpus cardiacum (CC) and released shortly after a blood meal[6]. Previous work had demonstrated that the ovary begins to produce ecdysone after a blood meal[8]. Considerable evidence suggests that the ecdysone, after being converted to 20-hydroxyecdysone, stimulates the synthesis and secretion of vitellogenin by the fat body[8-12]. The vitellogenin is taken up by the growing oocytes and becomes part of the yolk. Analysis of the amount of 20-hydroxyecdysone present in females after a blood meal showed that significant quantities were first seen 4–6 h after a blood meal[8]. Vitellogenin synthesis begins at about the same time[13]. The early decapitation experiments of Gillett demonstrated that the head was necessary for 4–8 h after a blood meal for egg development to proceed normally[3]. A similar critical time was found for the control of vitellogenin synthesis by the brain hormone[7]. The coincidence of this timing suggested to us that the ovary was the target of the brain hormone. The experiments reported here were designed to test the hypothesis that the brain hormone released after a blood meal acted on the ovary, causing the synthesis of ecdysone. Our results confirm this.

In earlier work we had successfully stimulated vitellogenin synthesis by incubating ecdysone-secreting ovaries with fat body preparations[7]. Our success with this *in vitro* approach prompted us to try to activate ovarian secretion of ecdysone by incubating ovaries with brains. Ecdysone in the medium after incubation was measured using an ecdysone radioimmunoassay[14]. We found that incubating whole brains (not including the CC, as it is removed with the aorta) with ovaries stimulates ecdysone secretion (Table 1). This effect is almost entirely due to the midbrain; other parts of the nervous system have little or no effect. Brains from adult males and from larvae also stimulate ovaries, although larval brains have half the activity of adult brains. Surprisingly, the CC and/or brains of several related insects activate ecdysone synthesis by mosquito ovaries (Table 1). The CC of larval cockroaches show considerably greater activity than those of adults. Cockroach CC is also much more active than brains and CC from a butterfly or the brains of a fly. The control of ovarian ecdysone secretion by a brain hormone in *Locusta* has been recently described[15] but in this case male or larval brain and CC were not stimulatory.

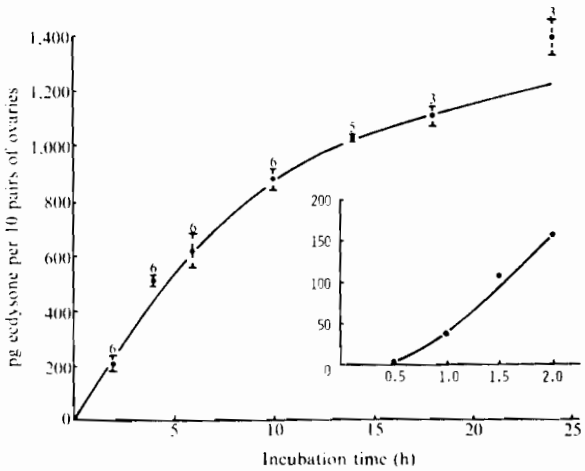

Fig. 1 Time course of ecdysone synthesis in response to head extract. 10 mg of heads were homogenised in 1 ml of sterile *Aedes* saline and treated as described in Table 2 legend except that the extract was not heated. The medium was assayed for ecdysone by RIA[14] after incubation. The data points represent the mean ± s.e.m. The number of replicates is indicated. The inset represents a

Table 1 Effect of parts of the central nervous system on ecdysone secretion by ovaries

Aedes aegypti (mosquito)	pg ecdysone per 10 pairs of ovaries ± s.e.m.
Adult female	
Whole brain	510 ± 68
Midbrain	339 ± 39
Optic lobes	0
Suboesophageal ganglia	16 ± 10
Thoracic ganglia	18 ± 0.9
Abdominal nerve cord	46 ± 6.9
Adult male	
Whole brain	496 ± 58
4th instar larva	
Whole brain	206 ± 17
Periplaneta americana (cockroach)	
Larval CC	744 ± 82
Adult CC	505 ± 52
Papilio polyxenes (butterfly)	
Larval brain and CC	163 ± 28
Adult brain and CC	149 ± 17
Musca domestica (fly)	
Adult brain	132 ± 18

Parts of the nervous system from five animals were frozen and thawed and incubated with ovaries from 10 female mosquitoes for 18 h at 25 °C in sterile *Aedes* saline[18] containing 0.15 mg ml^{-1} penicillin and 0.25 mg ml^{-1} streptomycin. This saline is isosmotic with haemolymph[19]. The adults used were 3–5 d old and were maintained on 3% sucrose at 27 °C. The medium and one rinse of the ovaries were combined and assayed for ecdysone using a radioimmunoassay (RIA)[14]. As we have previously shown that ovaries secrete only α-ecdysone when incubated *in vitro*[8], the results are expressed as pg ecdysone per 10 pairs of ovaries. The antiserum used was approximately equally sensitive to α-ecdysone and 20-hydroxyecdysone. Ovaries without any added nervous system gave a background of 40 pg of ecdysone that was subtracted from the above values in this experiment only. Each value represents the mean ± s.e.m. of three replicate studies. No correction is made in these data for the relative size of the brains used.

Saline extracts of whole mosquito heads also stimulate ecdysone production by the ovary. Ecdysone synthesis is linear for 10 h after exposure to head extracts, with a lag of 0.5 h (Fig. 1). In contrast, there is a delay of 6–8 h after a blood meal before the main peak of ecdysone is seen in the whole animal[8]. As the response *in vitro* is almost immediate (Fig. 1), this suggests that release of brain hormone *in vivo* is delayed. Gillett[3] came to the same conclusion by noting the effects of decapitation on egg development. The incubated ovaries are quite sensitive to the factor in head extracts: the amount of hormone present in 1.5–2 heads is sufficient to stimulate a half-maximal response in 10 pairs of ovaries incubated in 50 μl of extract (Fig. 2). At high concentrations of head extract the stimulation of ecdysone is

apparently due to a detrimental factor in the extract that is diluted out more rapidly than the hormone. Heating the extract has no effect on the inhibition.

Evidence that the ovary produces ecdysone *de novo* in response to the brain hormone was obtained by examining the amount of ecdysone in the ovaries and incubation medium before and after exposure to head extract (Table 2). A small amount of ecdysone is detectable in the ovary before the experiment. After 18 h with the head extract, 30 times more ecdysone is found in the incubation medium than in the ovary.

We have demonstrated that the ovary is indeed the target tissue of a brain hormone that stimulates ecdysone synthesis. This hormone is probably identical with the EDNH described by Lea[5,6], as the active factor is found in the region of the brain containing the medial neurosecretory cells (Table 1). A major unsolved problem concerns the nature of the stimuli that cause the release of EDNH after a blood meal. Neither the act of feeding nor stretching of the abdomen during feeding seems to be important[6]. There is some evidence that a humoral agent released after blood feeding is involved; this may be an early product of blood meal digestion[16].

EDNH is analogous in function to PTTH, the prothoracicotropic hormone found in immature insects. Both cause the synthesis of ecdysone by their respective target tissues; the function of the ecdysone in immature insects is to cause moulting. The similarity may be more than analogous, given that EDNH activity is present in larval mosquito brains and the brains and CC of several other insects. It is possible that these two hormones are structurally similar and therefore homologous to some degree. Work in our laboratory on the chemistry of EDNH has shown it to be a peptide of molecular weight about 6,500 with some similarities to PTTH from *Bombyx mori*[17].

The neuroendocrine control of ecdysone synthesis by the mosquito ovary resembles in several ways the control of oestrogen synthesis by the vertebrate ovary. In both, a peptide produced by neurosecretory cells or the pituitary stimulates the synthesis of a steroid by the ovary. Perhaps the cyclic production of eggs that occurs in both is best accomplished by this type of control mechanism.

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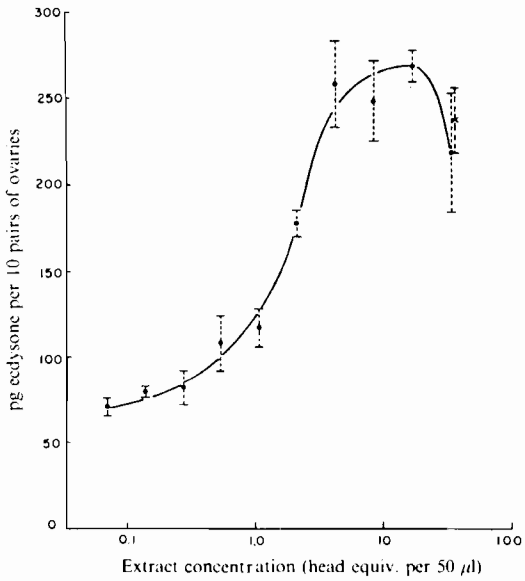

Fig. 2 Ecdysone synthesis by ovaries in response to increasing amounts of head extract. 20 mg of mosquito heads obtained as described in Table 2 legend were homogenised in 0.8 ml of sterile saline[18]. The extract was frozen and thawed and then centrifuged for 30 min at 10,000*g*. The sample was divided, one part was serially diluted and the other heated at 50 °C for 15 min. Ovaries from 10 7-d-old females were incubated with 50-μl samples of the extract for 6 h at 25 °C. Ecdysone was determined in the incubation medium by RIA[14]. The results were converted to head equivalents by the factor, 1 head = 36 μg. Data given are mean ± s.e.m. of five replicates. X Indicates the heat-treated sample.

Table 2 Synthesis of ecdysone by ovaries in response to head extract

	pg ecdysone per 10 pairs of ovaries ± s.e.m.
Ovaries before incubation	14
Saline extract before incubation	0
Ovaries after incubation	35
Saline extract after incubation	1,100 ± 25

Heads were obtained from 5–10-d old adult mosquitoes maintained on 3% sucrose by shaking animals frozen at −60 °C to break them apart and separating out the heads using US standard sieves. Heads were retained by a mesh size of 0.425 mm, lyophilised and 5 mg (36 μg per head) homogenised in 1 ml of sterile *Aedes* saline[18]. The extract was then sonified three times for 0.5 min and centrifuged for 5 min at 9,000*g*. The extract was heated at 50 °C for 15 min and centrifuged again. Heating effects some purification but does not significantly reduce the activity of the extract. Samples of supernatant (50 μl) were incubated with 3 groups of 10 pairs of ovaries each. After 18 h the ovaries were removed and combined to provide enough material for the RIA. Also 30 pairs of ovaries that were not incubated were dissected. The ovaries were homogenised and extracted with 50% methanol. The supernatant after centrifugation for 5 min at 9,000*g* was dried down and assayed for ecdysone by RIA[14]. The saline extract before and after incubation was also analysed for ecdysone. Ovary donors were as described in Table 1.

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