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GENERAL BIOLOGY

Octopamine Regulates the 20-Hydroxyecdysone Level in *Drosophila* Females

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Octopamine (OA), a biogenic amine of insects, arthropods, and mollusks, was first found in octopuses, which determined its name. In insects, OA functions as a neurotransmitter, neuromodulator, and neurohormone (for a review, see [1]). It was shown that, as a neurotransmitter, OA ensures neuromuscular transmission in oviduct and ovipositor muscles and is, therefore, involved in the regulation of the reproductive function of insects, regulating the processes of ovulation and ovipositing [2–4]. As a neurohormone, OA also controls the reproductive function of insects, regulating metabolism of the gonadotropic juvenile hormone (JH) [5–7].

When analyzing available published literature on the second insect gonadotropin, 20-hydroxyecdysone (20E), we did not find any paper regarding the effect of OA on the level of 20E in vivo. The only study was performed in vitro in larvae rather than adults: Hirashima et al. showed that an addition of OA to culture medium affected the synthesis of ecdysteroids by the prothoracic glands of *Bombyx mori* larvae [8].

However, our recent study of the effect of experimental increase in OA content on oogenesis and fecundity of wild-type *Drosophila melanogaster* and *Drosophila virilis* showed that feeding the flies with OA significantly decreased the number of vitellogenic (stages 8–10) and mature (stage 14) oocytes and drastically decreased fecundity of flies [9]. Since it is known that an increase (experimental or mutational) in the level of 20E causes degradation of early vitellogenic oocytes in *Drosophila* [10, 11], we assumed that the decrease in the fecundity of flies treated with OA in our experiments [9] might be due to an increase in the 20E titer in them.

In this study, we tested the above assumption and showed that (1) an experimental increase in the level of OA in wild-type *D. virilis* females causes an increase in the level of 20E and (2) the level of 20E is drastically decreased in mutant *D. melanogaster* females devoid of OA.

This study was performed with the wild-type *D. virilis* strain 101 and two *D. melanogaster* strains—the wild-type Canton S strain and the mutant strain $T\beta h^{nM18}$. Flies of the latter strain are devoid of OA as a result of null mutation at the gene for tyramine β-hydroxylase, an enzyme that converts tyramine into OA [2]. Cultures were grown in the standard nutrient medium at 25°C at a density of 20 larvae per 7 ml of nutrient medium. Cultures were synchronized twice by emergence of larvae and imagines (flies emerged within 3–4 h were collected). To treat *D. virilis* with octopamine, the flies immediately after emergence were placed into vials (three males and three females in each vial), the bottom and walls (1 cm) of which were covered with filter paper wetted with a solution (0.5 ml) containing 0.5% sucrose, 0.2% yeast, and 5 mg of OA. In the control series, the solution contained no OA. To determine the content of 20E, methanol extracts from 30 (in the case of *D. virilis*) or 50 (in the case of *D. melanogaster*) females were prepared. Ecdysteroids were isolated by solid-phase extraction using a Diapak C16 concentrating cartridges (BioKhimMak ST, Russia) and then chromatographed. Preliminary tests with the use of standard preparations of ecdysone, 20E, and [^{3}H]-ecdysone showed that the eluate contained 95–99% of ecdysteroids of the original sample. Chromatography was performed using an Agilent 1100 liquid chromatograph equipped with a Diasphere-110-C16 column (150 × 2 mm, particle diameter 5 μm; BioKhimMak ST, Russia) in the acetonitrile–water system (17 : 100) at a flow rate of 0.4 ml/min. Ecdysteroids were detected using a quadrupole mass spectrometer. The amount of ecdysteroids in samples was determined by comparing the peak areas of compounds of interest with the peak areas of standards. Statistical significance of results was estimated using Student's *t* test.

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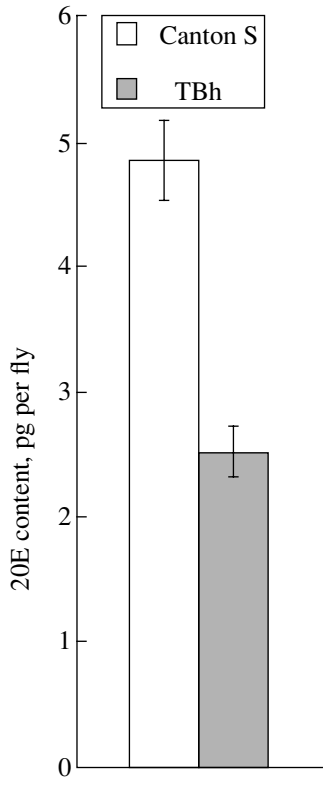

Fig. 1. Effect of mutation $T\beta h^{nM18}$ (TBh), leading to a complete absence of octopamine in *Drosophila*, on the level of 20-hydroxyecdysone (20E) in one-day-old *D. melanogaster* females. Canton S is a wild-type strain. Each value is the mean of five (Canton S) to ten (TBh) measurements.

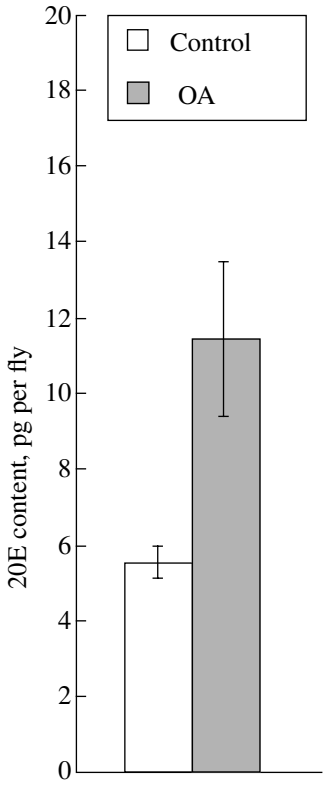

Fig. 2. Effect of experimental increase in octopamine (OA) level (feeding flies with octopamine) on the content of 20-hydroxyecdysone in two-day-old wild-type *D. virilis* females. Each value is the mean of five or six measurements.

Earlier, we showed that, in the $T\beta h^{nM18}$ females devoid of OA, the level of JH degradation is markedly increased compared to the wild-type Canton S females [7]. This means that the titer of JH hormone is decreased, because the processes of synthesis and degradation of JH in *Drosophila* are in antiphase and under a common control [12, 13]. On the other hand, it is known that JH stimulates the synthesis of 20E in *Drosophila* ovaries [14]. Therefore, it can be anticipated that the level of 20E in the females devoid of OA will be lower than in the wild-type females.

Figure 1 shows the results of measurement of 20E level in one-day-old $T\beta h^{nM18}$ females devoid of OA and Canton S females. It can be seen that, in the females devoid of OA, the level of 20E is drastically (by a factor of 2) decreased compared to the wild-type females (the differences were statistically different at $p < 0.001$).

We also showed earlier that feeding the wild-type females of the *D. virilis* strain 101 with OA resulted in a drastic decrease in JH degradation in them (i.e., in an increase in the titer of the hormone) [9]. If our assumption that OA affects 20E metabolism indirectly, via the JH metabolism system, is true, it can be anticipated that the treatment of strain 101 females with OA will lead to an increase in the 20E level in them.

Figure 2 shows the results of measurement of the 20E content in OA-fed and control two-day-old wild-type *D. virilis* females. It can be seen that an increase in the OA level in two-day-old females induces a change opposite to that observed in the absence of OA: the 20E level in the females administered with OA was twice as high as the hormone level in the control flies (the differences were statistically significant at $p < 0.01$).

Thus, the results obtained in this study indicate that OA regulates the level of 20E in *Drosophila* in vivo and that this regulation is apparently mediated by JH.

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