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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Effect of Octopamine on Ecdysone-20 Monooxygenase Activity in *Drosophila*

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The reproductive function of *Drosophila* females is regulated by two gonadotropic hormones—juvenile hormone (JH) and 20-hydroxyecdysone (20E). Immediately after *Drosophila* emergence, gonadotropins induce the synthesis of yolk proteins in the in the fat body and ovarian follicular cells, maintain it at a certain level after fertilization, and regulate absorption of yolk proteins by oocytes (for a review, see [1, 2]). 20E is synthesized in organs of the abdominal cavity in the form of the prohormone ecdysone (E), in target tissues, the latter is converted into the active form, 20E, as a result of hydroxylation by ecdysone-20-monooxygenase (E20MO), which is classified into the group of cytochrome P450-dependent monooxygenases (for a review, see [3]).

Earlier, we discovered that E20MO plays an important role in the regulation of the 20E titer in heat stress. Using a genetic model consisting of two *D. virilis* strains—the wild-type strain and the mutant strain differing from the wild-type one in the response of 20E to heat stress (38°C)—we showed that the 20E titer in *Drosophila* in stress is regulated at the level of conversion of E into 20E: the dynamics of E20MO activity in one-day-old female of each strain coincided with the dynamics of the 20E titer [4].

Taking into account the facts that the neuroendocrine stress response of insects, in addition to 20E and JH, is mediated by biogenic amines, octopamine (OA) and dopamine, whose content increases soon after the beginning of exposure to stressor [5], and that OA regulates the level of 20E (an increase in the content of OA results in an increase in the titer of 20E, and vice versa [6]), we assumed that OA may influence the titer of 20E by regulating the activity of E20MO.

The goal of this study was to test this assumption.

This study was performed with the wild-type *D. virilis* strain 101 and three *D. melanogaster* strains—the wild-type Canton S strain; the $T\beta h^{nM18}$ strain, devoid of OA as a result of null mutation at the gene for tyramine β-hydroxylase (Tβh) [7]; and the P845 strain, the precursor of the $T\beta h^{nM18}$ strain. Cultures were grown in the standard nutrient medium at 25°C at a density of 20 larvae per 7 ml of nutrient medium. The cultures were synchronized twice—by the emergence of larvae and adults (the flies emerged within 3–4 h were collected). To raise the OA level, wild-type *D. virilis* females were placed into vials (three flies per vial), the bottom and walls 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. After emergence, the flies were kept on this nutrient medium, whereupon the activity of

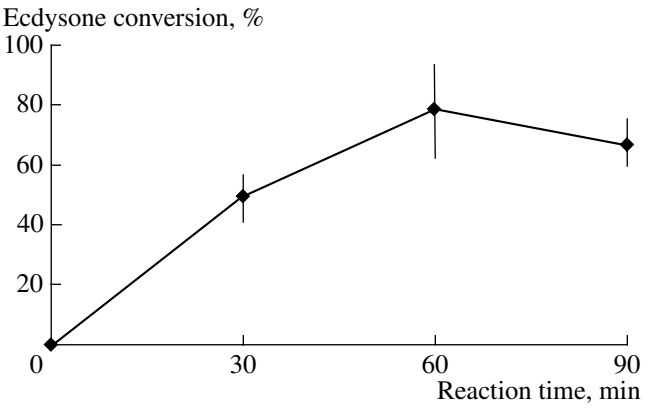

Fig. 1. Activity of ecdysone-20-monooxygenase in one-day-old wild-type *D. virilis* strain 101 females: dependence of conversion completeness on time. Each value is the mean of two measurements.

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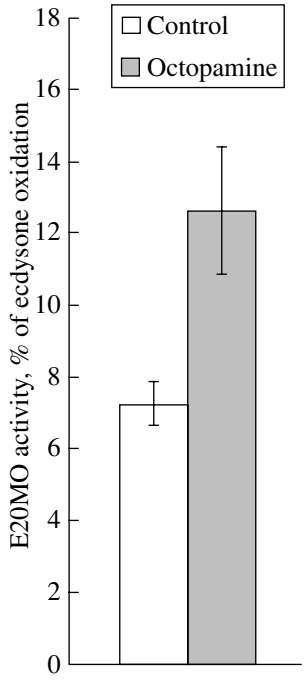

Fig. 2. Activity of ecdysone-20-monooxygenase in two-day-old wild-type *D. virilis* strain 101 females after feeding with octopamine (1% of oxidation ^{3}H-ecdysone corresponds to E20MO activity 0.11 pg/min per fly). Each value is the mean of 11–13 measurements.

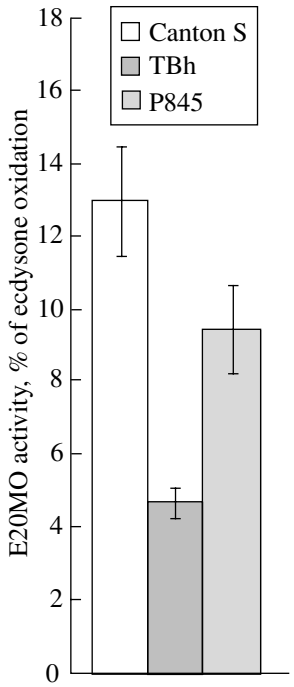

Fig. 3. Activity of ecdysone-20-monooxygenase in one-day-old females of *D. melanogaster* strains Canton S (CS, wild-type), $T\beta h^{nM18}$ (TBh, OA-devoid), and p845 ($T\beta h^{nM18}$ precursor). Each value is the mean of 6–9 measurements.

E20MO in them was determined. The control flies were fed with OA-free solution. The activity of E20MO was measured by the radiometric method combined with thin-layer chromatography (TLC), which was developed by Mitchell and Smith for *D. melanogaster* [8]. Three *D. virilis* females or five *D. melanogaster* females were used in the reaction. The zones of radioactive E and 20E on TLC plates were determined by cochromatography of standards—unlabeled E and 20E (Sigma, United States).

To make sure that, similarly to *D. melanogaster*, 30 min is the optimal reaction time for *D. melanogaster* as well, we studied the time course of the enzymatic reaction (Fig. 1a). It can be seen that the curve illustrating the conversion of E into 20E is linear within approximately 60 min. Based on this fact, we conducted the enzymatic reaction for 30 min for both *Drosophila* species.

Figure 2 shows the results of determination of E20MO activity in two-day-old wild-type *D. virilis* females that received OA for two days with food. As seen from this figure, an increase in the content of OA leads to a 1.7-fold increase in E20MO activity (differences from the control were significant at $p < 0.01$).

As mentioned above, mutant *D. melanogaster* strain $T\beta h^{nM18}$ females are completely devoid of OA [7]. Figure 3 shows the results of measurement of E20MO activity in one-day-old females of this strain in comparison to the females of the wild-type strain Canton S (CS) and strain p845, from which strain $T\beta h^{nM18}$ was obtained as a result of the *P* element transposition [7]. It can be seen that the activity of E20MO in the females lacking OA was 2.8 times lower than in the Canton S females and twice as low as in the p845 females (differences were significant at $p < 0.001$ in both cases). The last fact indicates that this effect is determined by the mutation as a result of which the flies lack the amine. The differences between Canton S and p845 females were nonsignificant.

Thus, the results of this study confirmed our assumption that OA regulates the titer of 20E at the level of its conversion from E, influencing the activity of the enzyme (E20MO) that catalyzes this reaction.

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