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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Juvenile Hormone and 20-Hydroxyecdysone Regulate *N*-Acetyltransferase Activity in *Drosophila virilis*

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It is believed that *N*-acetylation may be the main pathway of inactivation of biogenic amines [1–6]. For example, it was shown that the activity of *N*-acetyltransferase (aaNAT, NAT, EC 2.3.1.87) in *D. melanogaster* is much higher than the activity of monoamine oxidase, another enzyme involved in amine degradation [2, 6]. Similar results, obtained for imagoes of *Ostrinia nubilalis* [3] and *Periplaneta americana* [5], allowed the authors of these works to assume that NAT may be the key enzyme that catalyzes dopamine (DA) and octopamine (OA) degradation in insects.

Since NAT was first discovered in nervous tissue of *Drosophila* by Dewhurst et al. [2], these authors concluded that this enzyme is required not only for cuticle sclerotization but also for the synthesis and degradation of neurogenic catecholamines and their derivatives. This conclusion was confirmed later by other authors, who detected *N*-acetyltransferase activity not only in the cuticle and eggshell of insects but also demonstrated *N*-acetylation of biogenic amines in their nervous system [1, 5, 7].

Earlier, we showed that biogenic amines regulate the level of gonadotropins, juvenile hormone (JH), and 20-hydroxyecdysone (20E) in *D. virilis* and *D. melanogaster*: an increase in the content of DA and OA induces an increase in the level of 20E [8, 9] and JH (decreases its degradation) [10]. Note that this regulation is ensured by the feedback principle: an increase in the titer of JH in young *Drosophila* females leads to a drop in the level of DA [11], whereas an increase in the titer of 20E increases the content of DA [12].

The regulation of the OA and DA contents by gonadotropins can be controlled at the level of their synthesis, degradation, or the precursor pool control.

The goal of this study was to determine whether JH and 20E regulate the content of DA and OA by changing the activity of NAT, an enzyme degrades both amines.

This study was performed with the wild-type *D. virilis* strain 101. Cultures were grown on the standard nutrient medium at 25°C at a density of 20–30 larvae per 7 ml of nutrient medium. The cultures were synchronized by the emergence of adults; the flies that emerged within 3–4 h were collected. Then, one group of flies was fed for one day with a diet containing 20E. In this case, the flies were kept in a 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 60 μg of 20E (Sigma, United States) dissolved in 60 μl of ethanol. In the control, the nutrient medium was supplemented with 60 μl of ethanol without 20E. The other group of flies (one-day-old females) were treated with 2 μg of JH III (Sigma, United States)

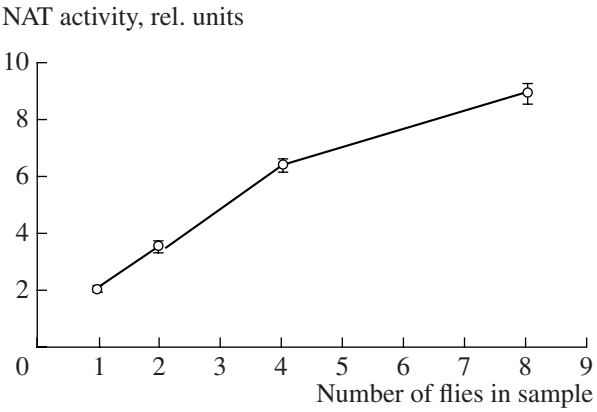

Fig. 1. Dependence of NAT activity on the enzyme concentration (determined by the number of flies containing the enzyme in sample). Each value is the mean of 5–18 repeated measurements.

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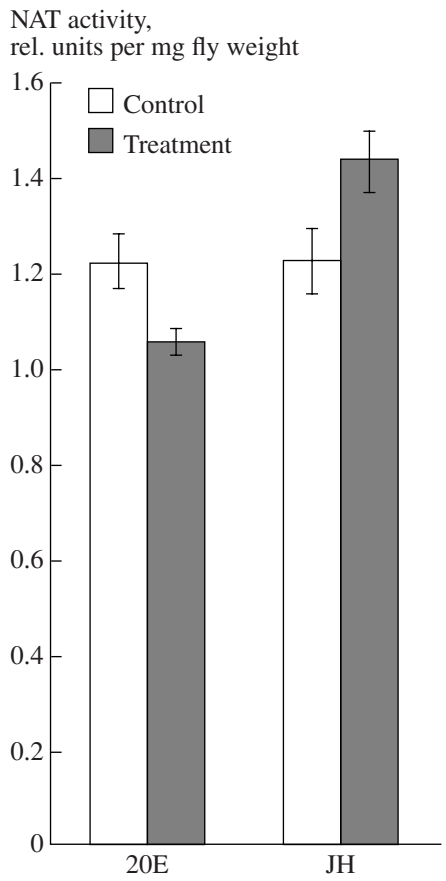

Fig. 2. Effect of feeding with 20-hydroxyecdysone (20E) and application of juvenile hormone (JH) on the acetylation level of dopamine in one-day-old wild-type *D. virilis* females. Each value is the mean of 7–13 repeated measurements.

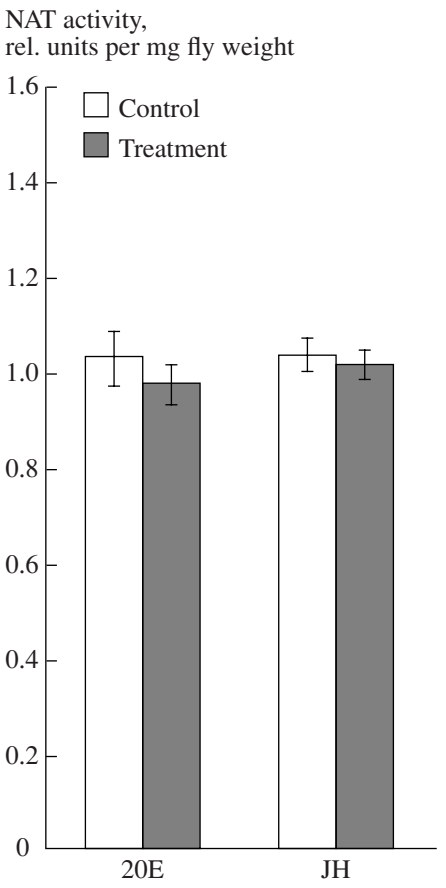

Fig. 3. Effect of feeding with 20-hydroxyecdysone (20E) and application of juvenile hormone (JH) on the acetylation level of octopamine in one-day-old wild-type *D. virilis* females. Each value is the mean of 7–10 repeated measurements.

dissolved in 1 μl of acetone. The control flies were treated with 1 μl of acetone. The activity of NAT was determined as described earlier [13]. The significance of results was estimated using Student's *t* test.

A special experiment was performed to determine the optimal amount of the enzyme required for measuring the activity of NAT in young *D. virilis* females. As seen from Fig. 1, the concentration of NAT in homogenates of one *D. virilis* fits the criterion of linearity of a calibration curve section.

Figure 2 shows the results of determination of NAT activity, with DA being used as a substrate, in one-day-old *D. virilis* strain 101 females 1 h after JH application. It can be seen that an experimentally induced increase in the titer of JH caused an increase in NAT activity (the differences from the control flies that were treated with acetone were statistically significant at $p < 0.01$). Thus, our data indicate that the decrease in the level of DA, caused by an increase in the titer of JH, which was discovered earlier [11], may be due to changes in the activity of NAT, the enzyme that catabolizes the amine.

Figure 2 also shows the results of measurement of NAT activity, with DA being used as a substrate, in one-day-old *D. virilis* strain 101 females after feeding them with 20E. It is well seen that an experimentally induced increase in the titer of 20E led to a marked decrease in NAT activity (the differences from the control were significant at $p < 0.05$). Therefore, the increase in the level of DA, following an increase in the titer of 20E, which was discovered in our previous work [8], may result, in particular, from a decrease in NAT activity.

To determine whether changes in the titer of gonadotropins influence the OA *N*-acetylation level, we measured the activity of NAT in one-day-old *D. virilis* females after JH application and feeding them with 20E, with OA being used as a substrate. The results of these experiments are shown in Fig. 3. It can be seen that the treatment with JH or 20E did not change significantly the OA acetylation rate. The fact that changed in the titer of gonadotropins correlate with changes in the acetylation rate of DA but not OA led us to assume that *D. virilis* contains at least two enzymes with *N*-acetyltransferase activity, which exhibit different substrate

specificity with respect to OA and DA. This assumption agrees with the data of other authors who reported the presence of various *N*-acetyltransferases in *P. americana, D. melanogaster*, and other insects [1, 5].

Thus, we showed that (1) juvenile hormone decreases the content of dopamine in young *Drosophila* females by increasing the activity of the enzyme that catalyzes its degradation, whereas 20-hydroxyecdysone increases the content of dopamine by decreasing the activity of this enzyme; and (2) the content of octopamine in them is regulated by gonadotropins at a level other than degradation.

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