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Effects of dopamine on juvenile hormone metabolism and fitness in *Drosophila virilis*

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Abstract

The effects of dopamine (DA) on juvenile hormone (JH) metabolism and fitness (estimated as fecundity and viability levels under heat stress (38 °C)) in *Drosophila virilis* have been studied. An increase of DA level obtained by feeding with DA reduced fitness of wild-type (wt) flies under stress, and decreased JH degradation in young wt females while increasing it in sexually mature wt females. A decrease in DA levels resulted from 3-iodo-tyrosine treatment and caused a decrease in JH degradation in sexually mature wt and heat sensitive (hs) mutant females (DA level in hs females is twice as high in wt females). A dramatic decrease in viability under stress and fecundity under normal conditions in wt, but not hs, females was observed. 3-iodo-tyrosine treatment also reduced the number of oocytes at stages 8–14, delayed oocyte transition to stage 10 and resulted in the accumulation of mature eggs in wt females. It delayed maturation of wt, but not hs, males as well, but did not affect their fertility. This advances our understanding of the regulation of JH metabolism by DA in *Drosophila* and suggests a crucial role for the basal DA level in fitness.

Keywords: Dopamine; Juvenile hormone; Reproduction; Survival under stress; *Drosophila virilis*

1. Introduction

It has long been known that biogenic amines, which control energy metabolism in insects (Candy, 1979; Downer, 1979a,b; Orchard et al., 1982; Woodring et al., 1989; Fields and Woodring, 1991) are important components of the insect adaptive neuroendocrine stress reaction. Under unfavorable conditions in various species (locusts, cockroaches, bees, beetles, fruit fly) the levels of octopamine (OA) and dopamine (DA) rise steeply in the hemolymph and in tissues of neural origin. The response is non-specific and it occurs when insects are exposed to high and low temperatures, mechanical and chemical stimuli, high population density, continuous light and immobilization (Orchard and Loughton, 1981; Davenport and Evans, 1984; Kozanek et al., 1986; Harris and Woodring, 1992; Rauschenbach et al., 1993; Hirashima and Eto, 1993; Hirashima et al., 1993a,b, 1994, 2000). It has also been established that metabolism of juvenile hormone (JH), which is known to control the reproductive function of insects (see reviews: Koeppe et al., 1985; Bownes et al., 1993), is regulated by biogenic amines. Thus, exogenous OA was shown to inhibit JH synthesis and its release from corpora allata in *Gryllus bimaculatus* and in females of *Diploptera punctata* (Thompson et al., 1990; Woodring and Hoffmann, 1994). On the other hand, OA was shown to stimulate JH release from the corpora allata in larvae and adults of worker bees, *Apis mellifera,* and in males of *Locusta*

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migratoria (Lafon-Cazal and Baehr, 1988; Kaatz et al., 1994; Rachinsky, 1994). Such different actions of OA on corpora allata may be due either to interspecies differences or to the diverse effects of OA on the JH system in different sexes and at different developmental stages. The existence of ontogenetic differences in the control of JH synthesis has been demonstrated for DA: in females of *Blattella germanica* the amine stimulates JH production and increases oocyte growth during days 1 and 2 of the first ovarian cycle and causes the opposite effect on days 6 and 7 (Pastor et al., 1991). Ontogenetic differences in the control of JH synthesis by DA has also been established for larvae of *Manduca sexta*: DA stimulates hormone biosynthesis in the corpora allata in the first two days of the last larval stage but inhibits the corpora allata on days 3 to 6, at the beginning of the prepupal stage (Granger et al., 1996). It was also established that biogenic amines may have a regulatory action not only on JH synthesis but also on its degradation: OA increased the activity of JH-esterase in larvae of *Bombyx mori* and pupae of *Tribolium freemani* (Hirashima et al., 1999).

We have previously established that in *Drosophila*, as in many other insect species, OA and DA regulate JH metabolism. The effect of DA had a distinctly developmental character, inhibiting JH degradation in the young non-ovipositing females and stimulating it in sexually mature females (Gruntenko et al., 2000, 2001). This regulation was shown to have a feedback since JH titer affected DA levels (Gruntenko et al., 2003b). We also revealed that the ability to survive unfavorable conditions correlated with DA content in *Drosophila* under normal conditions (DA basal level). DA content correlated negatively with the survival of flies under heat stress and positively with their survival under starvation (Gruntenko et al., 2004). We concluded that the genes that regulate DA metabolism must take part in the control of both insect reproductive function (mediated via JH system) and adaptability to unfavorable conditions of various types (Gruntenko and Rauschenbach, 2004). However, this conclusion was made on the basis of a comparison of JH metabolism, reproductive function and viability under stress in wild-type (wt) *Drosophila melanogaster* and *Drosophila virilis* strains compared with those observed in strains that carry mutations changing levels of the biogenic amines (Gruntenko et al., 2000, 2001, 2004). Because of this, we could not rule out completely the possibility that the observed effects could be due to action of other genes that we have not identified.

We report here that an experimental increase or decrease of DA content in wt *D. virilis* leads to changes in JH degradation levels in females and affects their fecundity and viability under heat stress.

2. Materials and methods

2.1. Maintenance of stocks

Two strains of *D. virilis* were used: 101, wt, and mutant line 147 (hs mutant), carrying mutations *brick*, *broken,* and *detached* on chromosome 2, a *temperature-sensitive* (heat stress hs) conditional larval lethal on chromosome 6 (Rauschenbach et al., 1984), and an X-linked mutation which sharply increases DA content in adults (Rauschenbach et al., 1993). Adult hs flies show decreased fertility under normal conditions and decreased viability under heat stress (Rauschenbach et al., 1993, 1996). Cultures were raised on standard medium (Rauschenbach et al., 1987) at 25 °C at a density of 20 larvae/7 ml of medium, and adults were synchronized at eclosion.

2.2. JH hydrolysis assay

In the present study we measured JH hydrolysis by the partition assay of Hammock and Sparks (1977), because we had previously shown that in *D. virilis* most JH degradation was carried out by JH esterase, and the activity of JH epoxide hydrolase was low and did not change under stress (unlike that of JH esterase which decreases steeply under various stress conditions) (Rauschenbach et al., 1995a; Khlebodarova et al., 1996). Each fly was homogenized in 30 μl ice-cold 0.1 M sodium-phosphate buffer, pH 7.4, containing 0.5 mM phenylthiourea. Sample size varied from 7 to 12 individuals for each group. Homogenates were centrifuged for 5 min at 13030 g, and samples of the supernatant (10 μl) were taken for the assay. A mixture consisting of 0.1 μg unlabeled JH-III (Sigma, additionally purified before use) and 12,500 dpm [3H]-JH-III labeled at C-10 (17.4 Ci/mmol, NEN Research Products, Germany) was used as a substrate. The reaction was carried out in 100 μl of the incubation mixture for 30 min, and was stopped by the addition of 50 μl of a solution containing 5% ammonia and 50% methanol (V/V), and 250 μl heptane. The tubes were shaken vigorously and centrifuged at 13030 g for 10 min. Samples (100 μl) of both organic and aqueous phases were placed in vials containing dioxane scintillation fluid and counted. Control experiments have shown a linear substrate–reaction product relationship; further the activity measured is proportional to the amount of supernatant (i.e. enzyme concentration) (Gruntenko et al., 1999, 2000).

2.3. DA content measurements

Flies were homogenized on ice in 0.1 M $HClO_4$. The homogenates were centrifuged for 10 min at 12,000 rpm. The supernatant was filtered through a nylon filter