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Dopamine and Octopamine Regulate 20-Hydroxyecdysone Level In Vivo in *Drosophila*

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The effects of increased level of dopamine (DA) (feeding flies with DA precursor, L-dihydroxyphenylalanine, L-DOPA) on the level of 20-hydroxyecdysone (20E) and on juvenile hormone (JH) metabolism in young (2-day-old) wild type females (the strain *wt*) of *Drosophila virilis* have been studied. Feeding the flies with L-DOPA increased DA content by a factor of 2.5, and led to a considerable increase in 20E level and a decrease of JH degradation (an increase in JH level). We have also measured the levels of 20E in the young (1-day-old) octopamineless females of the strain $T\beta h^{nM18}$ and in wild type females, *Canton S*, of *D. melanogaster*. The absence of OA led to a considerable decrease in 20E level (earlier it was shown that in the $T\beta h^{nM18}$ females, JH degradation was sharply increased). We have studied the effects of JH application on 20E level in 2-day-old *wt* females of *D. virilis* and demonstrated that an increase in JH titre results in a steep increase of 20E level. The supposition that biogenic amines act as intermediary between JH and 20E in the control of *Drosophila* reproduction is discussed. Arch. Insect Biochem. Physiol. 65:000–000, 2007.

KEYWORDS: *Drosophila*; 20-hydroxyecdysone; dopamine; octopamine; juvenile hormone

INTRODUCTION

It has long been established that juvenile hormone (JH) and ecdysteroids (ecdysone and 20-hydroxyecdysone (20E)) play a gonadotropic role in insect reproduction (Koeppe et al., 1985; Bownes, 1989; Raikhel et al., 2004). According to the model generally accepted, JH, synthesized by corpus allatum (CA), stimulates ecdysteroid synthesis in the ovaries. Ecdysteroids, produced by the ovarian follicular cells, stimulate vitellogenin (Vt) synthesis in the fat body; Vt is subsequently taken up from hemolymph by ovaries. The production of both hormones is under control of a third group of insect gonadotropins, neuropeptides (Postlethwait and Shirk, 1981; Bownes, 1989; Simonet et al., 2004). Richard et al. (1998, 2001) propose that in *Drosophila*, JH initiates only early stages of vitellogenesis in the fat body and in the ovarian follicular cells and it stimulates ecdysteroid production in the ovary, while 20E plays a prominent role in the control of oogenesis by stimulating the late stages of YP production in the fat body, their transportation from hemolymph to the nurse cells, and their further uptake by the oocytes. Soller et al. (1999), based on the results of experiments on the

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effect of exogenous JH and 20E treatment on *D. melanogaster* vitellogenesis, have come to the conclusion that the development of vitellogenic oocytes, including both YP production by the follicular cells and their uptake by the oocytes, is promoted by JH, while 20E regulates previtellogenic stages of the oocyte development. The authors also propose that for the normal progress of oogenesis in *Drosophila*, a proper balance between JH and 20E is of a paramount importance (Soller et al., 1999).

Recently, we obtained data (Rauschenbach et al., 2004a; Gruntenko et al., 2005a,b; Karpova et al., 2005) that support both the supposition by Soller et al. (1999) about the importance of the gonadotropin balance in the control of *Drosophila* oogenesis and the concept of Richard et al. (2001) regarding the prominent role of 20E in the hormonal control of the *Drosophila* female reproductive function. We have shown that (1) an inbalance of gonadotropins (shifting the balance either to the side of JH or 20E) leads to reproductive defects; (2) an experimental increase in 20E levels in *D. virilis* results in a decrease in JH degradation (that is, an increase of its titre); (3) a decrease in 20E titre in females of the strain *ecdysoneless*^{1} of *D. melanogaster* at the restrictive temperature leads to an increase in JH degradation; (4) the mediator in 20E action upon JH metabolism is dopamine (DA): an increase in 20E titre increases DA levels in young females of *D. virilis* and decreases them in the mature ones. This leads to a decrease in JH degradation in both (Rauschenbach et al., 2004a; Gruntenko et al., 2005a,b; Karpova et al., 2005).

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The effect of 20E on the metabolism of biogenic amines has also been shown in other insect species (Hiruma et al., 1985; Hiruma and Riddiford, 1990; Ferdig et al., 2000; Lehman et al., 2000; Mesce, 2002; Zufelato et al., 2004). However, in the available literature we found no research into the effects of biogenic amines on 20E levels in insects in vivo.

Here we report data that demonstrate that the biogenic amines, DA and octopamine (OA), regulate 20E levels in *Drosophila*, with JH as a possible mediator in this regulation.

MATERIALS AND METHODS

Maintenance of Stocks

Two species of *Drosophila* were used: *D. virilis* (wild type strain 101 (*wt*)) and *D. melanogaster* (wild type strain *Canton S* and octopamineless strain $T\beta h^{nM18}$, carrying a null mutation at the *Tyramine β-hydroxylase* (*Tβh*) locus on X chromosome (Monastirioti et al., 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.

L-DOPA and JH Treatments of the Flies

Three newly eclosed females and 3 males were placed in vials in which the bottom and 1 cm of the wall were covered with filter paper soaked with 0.5 ml of culture medium (0.5% sucrose and 0.2% yeast in water). In the experimental series, 5 mg L-dihydroxyphenylalanine (L-DOPA) (Sigma) were added to this solution. Flies were transferred to vials with fresh medium daily.

To determine whether changes in JH titre could affect 20E level, we treated 2-day-old *wt* females with 0.2 μg JH-III (Fluka, Buchs, Switzerland) dissolved in acetone. Control females were treated with acetone (0.5 μl). 20E content was measured 12 h after application.

JH Hydrolysis Assay

JH hydrolysis was measured by the partition assay of Hammock and Sparks (1977). Each fly was homogenized in 30 μl of ice-cold 0.1 M sodium-phosphate buffer, pH 7.4, containing 0.5 mM phenylthiourea. Sample size varied from 5 to 8 individuals for each group. Homogenates were centrifuged for 5 min at 13,030*g*, and samples of the supernatant (10 μl) were taken for the assay. A mixture consisting of 0.1 μg of the unlabeled JH-III (Fluka, Buchs, Switzerland) and 12,500 dpm [3H]-JH-III labelled at C-10 (17.4 Ci/mmol, NEN Research Products, Rodgau-Jugesheim, Germany, additionally purified before use) was used as a sub-

strate. 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, 50% methanol (V/V), and 250 µl of heptane. The tubes were shaken vigorously and centrifuged at 13,030*g* for 10 min. Samples (100 µl) of both organic and aqueous phases were placed in vials containing dioxane scintillation fluid; radioactivity was estimated by liquid scintillation counting (Rackbeta 1209 counter, Vellag, Turku, Finland, at 67% counting efficiency for tritium). Control experiments have shown a linear substrate-reaction product relationship; the activity measured is proportional to the amount of supernatant (i.e., enzyme concentration) (Gruntenko et al., 1999, 2000).

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DA Content Measurements

Flies were homogenized on ice in 0.1 M $HClO_4$. The homogenates were centrifuged for 10 min at 13,030*g*. The supernatant was filtered through a nylon filter (Schleicher & Schuell, Spartan 3 mm/0.45 µm, Dassel, Germany) and 10 µl was injected directly into an HPLC column through a valve fitted with a 20-µl sample loop. Chromatography was carried out in a C16 reverse-phase column (Diaspher, 110-C16, 2.1 × 150 mm, 5 µm average particle size, BioChemMak, Russia) using an Agilent 1100 HPLC system, with a quaternary pump (including vacuum degasser) and thermo-controlled column compartment. Separated compounds were detected simultaneously by a variable wavelength detector (10-mm path length, 13-µl cell volume) set at 196 nm. Signals from the UV detector were recorded and integrated by a PC using the manufacturer's software. The flow rate was maintained at 0.4 ml/min, the mobile phase consisted of 0.025 M KH_2PO_4 (pH 3.0) buffer, 0.3 mM sodium heptanesulfonate as an ion-pair reagent, and 1.5% (v/v) acetonitrile. The concentration of DA was calculated by comparing the peak area between the sample and standard. The identities of the DA and tyrosine peaks in *Drosophila* samples were confirmed on the basis of comparison of their retention times with those of the standard mixture. UV-spectra of the peaks in *Drosophila* samples corresponded to UV-spectra of tyrosine and DA. Moreover, we added excess quantities of DA and tyrosine (20 ng and 100 ng, respectively) to some *Drosophila* samples to register the changes of heights of the appropriate peaks. Sample size varied from 5 to 6 measurements for each group.

20E Content Measurements

Flies (30 *D. virilis* individuals or 50 *D. melanogaster* ones) were homogenized in 500 µl of 100% methanol containing 400 pg of 25-S-inokosterone (kindly provided by Prof. V. Volodin, Institute of Biology, Russian Academy of Sciences, Siktivkar, Russia) as internal standard. Homogenate was heated in a water-bath (60°C) for 10 min and centrifuged at 13,030*g* for 10 min; supernatant was transferred to a glass tube. Residue was extracted repeatedly by 500 µl of methanol and the extracts were combined. One milliliter of the final extract was diluted with 4 ml of the double-distilled water and extracted twice by 5 ml of chloroform in a separating funnel. Water-methanol phase was applied onto a 3-ml disposable C16 extraction column (Diapack-C16, BioChemMak ST, Moscow, Russia). The fluid was drawn through the column using a 5-ml syringe, and ecdysteroids were eluted from the extraction column with 5 ml 65% methanol. The eluate was rotary concentrated to dryness at 60°C and resuspended twice in 500 µl of 100% methanol. The final methanol extract was concentrated to 50 µl, and 10 µl was injected onto an HPLC C16 column (Diaspher-C16, BioChemMak ST, Moscow, Russia, 2 × 150 mm, pore size 110A, particle size 5 µm), the mobile phase was 15% acetonitrile in water and the flow rate was 0.5 ml/min. 20-hydroxyecdysone and 25-S-inokosterone concentrations were determined by HPLC with a quadrupole mass-spectrometric detection system (Agilent 1100 Series LC/MSD VL, Palo Alto, CA) in the SIM mode according to Wainwright et al. (1997). Sample size varied from 5 to 6 measurements for each group.

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Statistical Analysis

The significance of the differences between the data sets was tested by the Student's *t*-test

RESULTS

Effect of Feeding Wild Type *D. virilis* Females With DA Precursor, L-DOPA, on DA Content, JH Degradation, and 20E Level

Figure 1 shows the results of measurement of DA content (A), JH degradation level (B) and 20E content (C) in the L-DOPA-fed and control 2-day-old *wt* females. The females fed with L-DOPA were much higher in DA content by a factor of 2.5 (differences from the control group are significant at $P < 0.001$).

We measured JH degradation level upon feeding the flies with L-DOPA. From Figure 1B, we notice that with DA increased by a factor of 2.5, JH degradation drops by a factor of one and a half (differences from control are significant at $P < 0.001$).

Feeding the flies with L-DOPA resulted in the increase of 20E level by a factor of 1.6 (Fig. 1C) (differences from the control group are significant at $P < 0.001$).

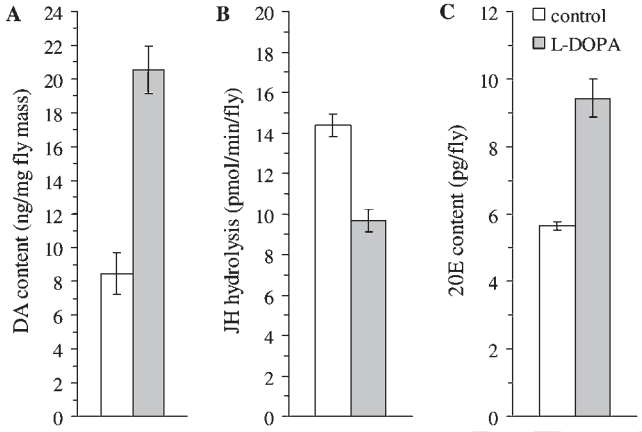

Fig. 1. Effect of L-DOPA feeding (5 mg per vial with 6 flies) on DA content (**A**), JH degradation (**B**), and 20E titre (**C**) in 2-day-old *wt* females of *D. virilis*. Means ± SE. Each value is an average of 5 to 8 (JH degradation) or 5 to 6 (DA and 20E) measurements.

JH Degradation and 20E Level in Octopamineless Females of *D. melanogaster* $T\beta h^{nM18}$ Strain

Figure 2 depicts the results of measurements of JH hydrolysis (Fig. 2A, reproduced from Gruntenko et al., 2000) and 20E levels (Fig. 2B) in 1-day-old females of the strain $T\beta h^{nM18}$, octopamineless as a result of a null mutation of the gene *tyramine β-hydroxylase* (Monastirioti et al., 1996) that converts tyramine into OA (Wright, 1987) and in females of a wild type strain, *Canton S*.

JH degradation level is steeply increased and 20E level is decreased in the octopamineless females in comparison with wild type females (differences between the strains are significant at $P < 0.001$ for both features).

The Effect of JH Titre Increase on 20E Level in *wt* Females of *D. virilis*

Figure 3 shows the results of measurement of 20E content in 2-day-old *wt* females after the ap-

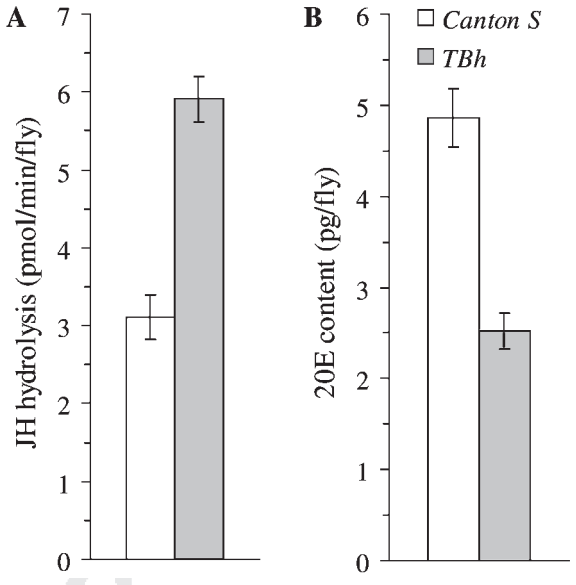

Fig. 2. Effect of $T\beta h^{nM18}$mutation (TBh) that leads to complete loss of OA on JH degradation (**A**, reproduced from Gruntenko et al., 2000) and 20E titre (**B**) in 1-day-old females of *D. melanogaster. Canton S* wild type strains. Means ± SE. Each value is an average of 5 (*Canton S*) to 10 (TBh) measurements.

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plication of JH-III dissolved in acetone and in the control treated with acetone.

The increase in JH titre results in a sharp increase of 20E level (differences from control are significant at $P < 0.001$).

DISCUSSION

Earlier we studied levels of JH degradation in females with a twofold increase of the DA content. The *D. melanogaster* strains *ste* and *ebony* carry a mutation that drastically decreases activity of the enzymes converting DA into *N*-β-alanyldopamine (Perez et al., 1997). We found that young females of both strains have considerably lower JH degradation levels and the mature flies have higher levels compared to wild type, *Canton S* (Gruntenko and Rauschenbach, 2004). We also showed that feeding flies of *wt* strain of *D. virilis* with DA resulted in a decrease in JH degradation in young (nonovipositing 2-day-old) females, while in the mature (7-day-old) ones, it led to an increase in JH degradation (Gruntenko et al., 2005b).

The results obtained in the present study when measuring JH degradation levels in 2-day-old L-DOPA-fed *wt* females agree with the above data: an increase in DA content (see Fig. 1A) leads to a decrease in JH degradation level (see Fig. 1B), which we infer to be indicative of an increase in JH level.

In wild type females of *D. melanogaster*, the regulation of JH synthesis and degradation tends to be opposing: both JH titre (Bownes and Rembold, 1987; Sliter et al., 1987) and JH synthesis (Altaratz et al., 1991) in young (1-day-old) wild type *D. melanogaster* females were substantially higher than in mature (5–6-day-old) flies. At the same time, JH degradation in young wild type *D. melanogaster* females is significantly lower than in the mature ones (Gruntenko et al., 2000, 2003). Females of the mutant strain *apterous*^{56f} of *D. melanogaster* were shown to have dramatically decreased JH synthesis (Altaratz et al., 1991) and sharply increased JH degradation (Gruntenko et al., 2003b). Considering all the above, we have suggested (Gruntenko et al., 2003) that (1) JH synthesis and degradation are under a common control system in the adult females of *Drosophila*, and (2) the factors stimulating the hormone synthesis inhibit its degradation and vice versa. This notion agrees well with the fact that an experimental increase of the JH titre in *wt* females of *D. virilis* leads to a decrease in its degradation (Rauschenbach et al., 2004a). The idea of the correlated regulation of JH synthesis and degradation in insects is also supported by the data of Renucci et al. (1990) showing that ovariectomy of *Acheta domesticus* females results in the simultaneous decrease of JH synthesis and increase in the activity of JH-esterase that degrades the hormone. In microarray experiments (Terashima and Bownes 2005), treatment of *D. melanogaster* starved females with JH leads to a down-regulation of JH-epoxide hydrolase 3 (the main JH-hydrolizing enzyme in adults females of *D. melanogaster*) (Khlebodarova et al., 1996).

As mentioned above, we have shown earlier that an increase in 20E level in young *wt D. virilis* females leads to an increase in DA content, and in sexually mature ones, to its decrease (Gruntenko et al., 2005a). In that case and if there is a feedback regulation (a direct effect of DA on 20E metabolic system), an increase in DA content in young

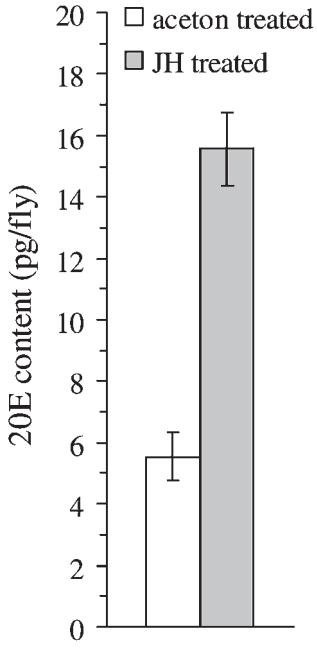

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Fig. 3. 20E titre (B) in 2-day-old *wt* females of *D. virilis* 12 h after the application of 2 μg of JH-III dissolved in acetone (controls were treated with acetone). Means ± SE. Each value is an average of 5 to 6 measurements.

females should result in a decrease in 20E level. Data presented in Figure 1C indicate that this is not the case: the 20E level is increased in young females with an increased DA content. At the same time, a rise in JH level (a decrease of its degradation) produced in young *Drosophila* females by the increase in DA content (Fig. 1A,B) should lead to a rise of 20E because JH activates ecdysone synthesis in ovaries of young females (Postlethwait and Shirk, 1981; Kelley, 1994; Simonet et al., 2004). Data in Figure 3 correlate with this: in JH-treated *wt* females, the 20E level is dramatically increased. Thus, we propose that DA has an effect on 20E metabolism, but this effect is indirect and mediated through the JH metabolic system.

Earlier we also studied levels of JH degradation in females of *D. melanogaster* octopamineless strain $T\beta h^{nM18}$ (Gruntenko et al., 2000). Both young and mature octopamineless females have JH degradation levels much higher (JH levels much lower) than those in wild type, *Canton S*, flies (Gruntenko et al., 2000; see Fig. 2A). If OA, like DA, regulates 20E through the JH metabolic system, one could expect octopamineless females to have 20E level lower than in wild type. The data of Figure 2B suggest that this is the case. The supposition that OA regulates 20E level indirectly via the JH metabolic system agrees with the results of our experiment in which OA content was increased by feeding *wt* females of *D. virilis* with the amine (Gruntenko et al., 2006): JH degradation decreased (JH level went up) and 20E level increased in the OA-treated females.

Summarizing the results of the present study and our previous data, we propose the following scheme of the reciprocal regulation of biogenic amines and gonadotropins in *Drosophila* (Fig. 4). DA increases JH level (inhibits JH degradation and apparently stimulates synthesis) in young females (see Fig. 1A; Gruntenko et al., 2005b) and decreases it (stimulates degradation and apparently inhibits synthesis) in sexually mature flies (Gruntenko and Rauschenbach, 2004; Gruntenko et al., 2005b). There is a feedback in this regulation; a rise in JH level leads to a decrease in DA content in young females and its rise in the mature ones (Gruntenko et al., 2003; Rauschenbach et al., 2004b). OA leads to a rise of JH level (inhibits JH degradation and, evidently, stimulates its synthesis) in young and mature females (see Fig. 2A, Gruntenko et al., 2000, 2006). 20E regulates JH indirectly via the DA metabolic system; a rise in 20E level increases DA content in young and decreases it in mature females, thus leading to a decrease of JH degradation (a rise in its titre) in both (Gruntenko et al., 2005a). DA influences 20E level indirectly via the JH metabolic system (see Fig. 1). OA is also likely to regulate 20E indirectly via the JH metabolic system (see Figs. 2,3).

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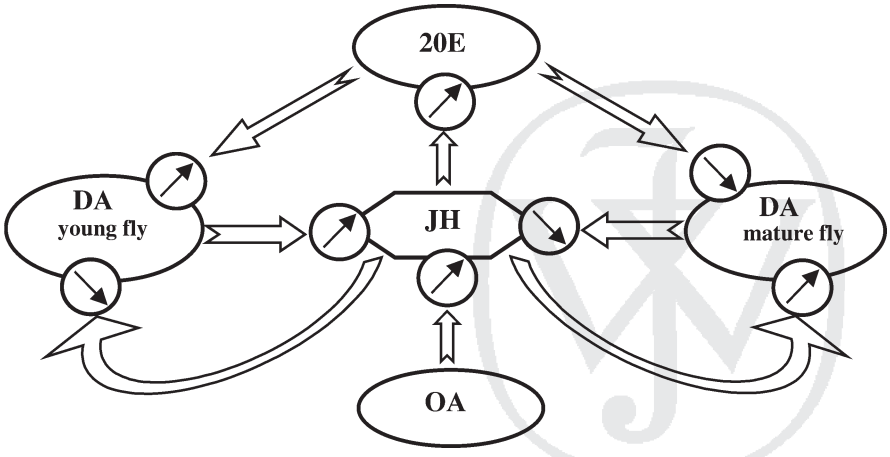

Fig. 4. Scheme of the reciprocal regulation of gonadotropins (JH and 20E) and biogenic amines (DA and OA) in *Drosophila*.

LITERATURE CITED

Altaratz M, Applebaum ShW, Richard DS, Gilbert LI, Segal D. 1991. Regulation of juvenile hormone synthethis in wild-type and *apterous* mutant *Drosophila*. Mol Cell Endocrinol 81:205–216.

Bownes M. 1989. The roles of juvenile hormone, ecdysone and the ovary in the control of *Drosophila* vitellogenesis. J Insect Physiol 35:409–413.

Bownes M, Rembold H. 1987. The titre of juvenile hormone during the pupal and adult stage of the life cycle of *Drosophila melanogaster*. Eur J Biochem 164:709–712.

Ferdig MT, Taft AS, Smartt CT, Lowenberger CA, Li J, Zhang J, Christensen BM 2000. *Aedes aegypti* dopa decarboxylase: gene structure and regulation. Insect Mol Biol 9:231–239.

Gruntenko NE, Rauschenbach IYu. 2004. Adaptive value of genes controlling the level of biogenic amines in *Drosophila*. Russ J Genet 40:703–709.

Gruntenko NE, Wilson TG, Monastirioti M, Rauschenbach IYu. 2000. Stress-reactivity and juvenile hormone degradation in *Drosophila melanogaster* strains having stress-related mutations. Insect Biochem Mol Biol 30:775–783.

Gruntenko NE, Chentsova NA, Andreenkova EV, Bownes M, Segal D, Adonyeva NV, Rauschenbach IYu. 2003. Stress response in a juvenile hormone deficient *Drosophila melanogaster* mutant *apterous*^{56f}. Insect Mol Biol 12:353–363.

Gruntenko NE, Karpova EK, Adonyeva NV, Chentsova NA, Faddeeva NV, Alekseev AA,. Rauschenbach IYu. 2005a. Juvenile hormone, 20-hydroxyecdysone and dopamine interaction in *Drosophila* virilis reproduction under normal and nutritional stress conditions. J Insect Physiol 51:417–425.

Gruntenko NE, Karpova EK, Alekseev AA, Chentsova NA, Saprykina ZV, Bownes M, Rauschenbach IYu. 2005b. Effects of dopamine on juvenile hormone metabolism and fitness in *Drosophila virilis*. J Insect Physiol 51:959–968.

AQ4 Gruntenko NE, Karpova EK, Alekseev AA, Chentsova NA, Bogomolova EV, Bownes M, Rauschenbach IYu. 2006. Effects of octopamine on reproduction, juvenile hormone metabolism, dopamine and 20-hydroxyecdysone contents in *Drosophila*. Arch Insect Biochem Physiol.

Hammock BD, Sparks TC. 1977. A rapid assay for insect juvenile hormone esterase activity. Anal Biochem 82:573–579.

Hirashima A, Hirokado S, Ohta H, Suetsugu E, Sakaguichi M, Kuwano E, Taniguchi E, Eto M. 1999. Titres of biogenic amines and ecdysteroids: effect of octopamine on the production of ecdysteroids in the silkworm *Bombyx mori*. J Insect Physiol 45:843–851. AQ5

Hiruma K, Riddiford LM. 1990. Regulation of dopa decarboxylase gene expression in the larval epidermis of the tobacco hornworm by 20-hydroxyecdysone and juvenile hormone. Dev Biol 138:214–224.

Hiruma K, Riddiford LM, Hopkins TL, Morgan TD. 1985. Roles of dopa decarboxylase and phenoloxidase in the melanization of the tobacco hornworm and their control by 20-hydroxyecdysone. Comp Physiol [B] 155:659–669.

Karpova EK, Gruntenko NE, Rauschenbach IYu. 2005. The *ecdysoneless*^{1} gene regulates metabolism of the juvenile hormone and dopamine in *Drosophila melanogaster*. Russ J Genet 41:1480–1486

Kelley TJ. 1994. Endocrinology of vitellogenesis in *Drosophila melanogaster*. Perspectives in Comparative Endocrinology. National Research Council of Canada. p 282–290 AQ6

Khlebodarova TM, Gruntenko NE, Grenback LG, Sukhanova MZ, Mazurov MM, Tomas BA, Hammock BD, Rauschenbach IYu. 1996. A comparative analysis of juvenile hormone metabolysing enzymes in two species of *Drosophila* during development. Insect Biochem Mol Biol 26:829–835.

Koeppe JK, Fuchs M, Chen TT, Hunt LM, Kovalick GE. 1985. The role of juvenile hormone in reproduction. In: Kerkut GA, Gilbert LI, editors. Comprehensive insect physiology, biochemistry and pharmacology, Vol. 8. New York: Pergamon Press. p 165–203.

Lehman HK, Klukas KA, Gilchrist LS, Mesce KA. 2000. Steroid regulation of octopamine expression during metamorphic development of the moth *Manduca sexta*. J Comp Neurol 424:283–296.

Mesce KA. 2002. Metamodulation of the biogenic amines: second-order modulation by steroid hormones and amine cocktails. Brain Behav Evol 60:339–349.

Monastrioti M, Linn CE, White K. 1996. Characterization of *Drosophila Tyramine β-hydroxylase* gene and isolation of mutant flies lacking octopamine. J Neurosci 16:3900–3911.

Perez M, Castillo-Marin N, Quesada-Allue LA. 1997. *N*-β-alanyl-dopamine synthase in *Drosophila melanogaster* and *Cer-* AQ7

atitis capitata melanic mutants. Drosoph Inf Serv 80: 39–41.

Postlethwait JH, Shirk PD. 1981. Genetic and endocrine regulation of vitellogenesis in *Drosophila*. Am Zool 21:687–700.

AQ8 Raikhel AS, Brown MR, Belles X. 2004. Hormonal control of reproductive processes. In: Gilbert LI, Klatrou K, Gill S, editors. Comprehensive molecular insect science. 3:433–491.

Rauschenbach IY, Lukashina NS, Maksimovsky LF, Korochkin LI. 1987. Stress-like reaction of *Drosophila* to adverse environmental factors. J Comp Physiol 157:519–531.

Raushenbach IY, Gruntenko NE, Bownes M, Adonieva NV, Terashima J, Karpova EK, Faddeeva NV, Chentsova NA. 2004a. The role of juvenile hormone in the control of reproductive function in *Drosophila virilis* under nutritional stress. J Insect Physiol 50:323–330.

Rauschenbach IYu, Gruntenko NE, Chentsova NA, Adonyeva NV, Karpova EK. 2004b. Feedback in the regulation of juvenile hormone titre by biogenic amines in *Drosophila*. Dokl Biol Sci 397:324–325

Renucci M, Strambi C, Strambi A, Augier R, Charpin P. 1990. Ovaries and regulation of juvenile hormone titer in *Acheta domesticus* L. (*Ortoptera*). Gen Comp Endocrinol 78:137–149.

Richard DS, Watkins NL, Serafin RB, Gilbert LI. 1998. Ecdysteroids regulate yolk protein uptake by *Drosophila melanogaster* oocytes. J Insect Physiol 44:637–644.

Richard DS, Jones JM, Barbarito MR, Cerula S, Detweiler JP, Fisher SJ, Brannigan DM, Scheswohl M. 2001. Vitellogenesis in diapausing and mutant *Drosophila melanogaster*: further evidence for the relative roles of ecdysteroids and juvenile hormones. J Insect Physiol 47:905–913.

Simonet G, Poels J, Claeys I, Van Loy T, Franssens V, De Loof A, Vanden Broeck J. 2004. Neuroendocrinological and molecular aspects of Insect reproduction. J Neuroendocrinol 16:649–659.

Sliter TJ, Sedlak BJ, Baker FC, Schooley DA. 1987. Juvenile hormone in *Drosophila*. Identification and titer determination during development. Insect Biochem 17:161–165.

Soller M, Bownes M, Kubli E. 1999. Control of oocyte maturation in sexually mature *Drosophila* females. Dev Biol 208:337–351.

Terashima J, Bownes M. 2005. A microarray analysis of genes involved in relating egg production to nutritional intake in *Drosophila melanogaster*. Cell Death Differ 12:429–40.

Wainwright G, Prescott MC, Lomas LO, Webster SG, Rees HH. 1997. Development of a new high-performance liquid chromatography-mass spectrometric method for the analysis of ecdysteroids in biological extracts. Arch Insect Biochem Physiol 35:21–31.

Wright TRF. 1987. Genetic of biogenic amines metabolism, sclerotisation and melanisation in *Drosophila melanogaster*. Adv Genet 24:127–221.

Zufelato MS, Lourenco AP, Simoes ZL, Jorge JA, Bitondi MM. 2004. Phenoloxidase activity in *Apis mellifera* honey bee pupae, and ecdysteroid-dependent expression of the prophenoloxidase mRNA. Insect Biochem Mol Biol 34:1257–1268.