Chapter 12
20-Hydroxyecdysone, Juvenile Hormone and Biogenic Amines: Mechanisms of Interaction in Control of *Drosophila* Reproduction Under Normal and Stressful Conditions

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Abstract Juvenile hormone (JH) and ecdysteroids play a gonadotropic role in insect reproduction. For the normal progress of oogenesis, a proper balance between JH and 20-hydroxyecdysone (20E) is of a paramount importance. An imbalance of gonadotropins (shifting the balance either to the side of JH or 20E) leads to reproductive defects: a rise in JH titre leads to oviposition arrest, a rise in 20E level, to the degradation of vitellogenic oocytes. Upon a change in the level of one of the gonadotropins as a result of a mutation, effect of a stressor or pharmacological agent, the balance is restored owing to the relative change in the titre of the other one. Mediators in the JH and 20E interrelationship are biogenic amines, dopamine and octopamine. Existence of the mechanism of gonadotropin's – reciprocal regulation is adaptive.

Keywords Drosophila • stress • reproduction • adaptability • Juvenile hormone • 20-hydroxyecdysone • dopamine • octopamine

12.1 Introduction

It has long been established that juvenile hormone (JH) and ecdysteroids (ecdysone and 20-hydroxyecdysone (20E)) play a gonadotropic role in insect reproduction (reviewed by 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 (Vg) synthesis in the fat body; Vg is subsequently taken up from hemolymph by ovaries. The production of both hormones is under control of a third group of insect gonadotropins, neuropeptides (reviews: 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

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production in the ovary, while 20E plays a prominent role in the control of oogenesis by stimulating the late stages of yolk proteins (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 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).

We obtained data (Gruntenko et al., 2003a, b, 2005a, b, 2007; Rauschenbach et al., 2004a, b, 2007; Karpova et al., 2005) which (i) 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, and (ii) demonstrate that in *Drosophila* there is a mechanism of reciprocal regulation of JH and 20E which is responsible for their proper balance. Here we present the data in short.

12.2 Imbalance of 20E and JH Leads to Reproductive Defects

To determine the effects of shifting the balance either to the side of JH or 20E on *Drosophila* reproduction we used two approaches: (1) studied the effect of stress, which changes sharply levels of JH and 20E (Rauschenbach et al., 1995; Hirashima et al., 2000; Gruntenko et al., 2003b), on oogenesis in wild type females of *D. virilis* (*wt* strain) and in females carrying the mutation that prevents the change in JH level under heat stress (*hs* strain) (Gruntenko et al., 2003b; Rauschenbach et al., 2004a); (2) estimated the influence of exogenous JH and 20E on fertility of females of *wt* strain (Rauschenbach et al., 2004a; Gruntenko et al., 2005a).

We first described the *hs* mutant phenotype as a temperature-sensitive conditional larval lethal. Mortality results from the absence of a response of the JH metabolic system to heat stress. *Wt* larvae enduring environmental stress (heating, crowding, starvation) are able to undergo normal metamorphosis because the factor that activates the JH-degrading enzyme in the hemolymph is inhibited. As a result, JH titre decreases slowly and JH activates ecdysone synthesis in the peritracheal gland in the absence of protoracicotropic hormone, the synthesis of which is inhibited under heat stress (Rauschenbach et al., 1984, 1987). The brain factor that activates the enzymes causing JH degradation is not inhibited under heat stress in *hs* larvae. As a result, JH is degraded, the peritracheal gland remains inactive, and a very low level of 20E prevents mutant larvae from undergoing metamorphosis, which leads to their death (Rauschenbach et al., 1984, 1987).

Wt adult females respond to heat stress (38°C) by an increase in the level of 20E and by a decrease in JH degradation level (Table 12.1). We believe that the latter is

Table 12.1 Effect of heat stress (38°C, 3 h) on JH degradation and 20E content in 1-day-old *D. virilis* females of *wt* and *hs* strains (Hirashima et al., 2000; Gruntenko et al., 2003b)

	JH degradation (pmol/min/fly)		20E content (pg/fly)	
	Control	Heat stress	Control	Heat stress
wt strain	13.7 ± 0.64	8.12 ± 0.67	10.7 ± 0.48	15.6 ± 1.05
hs strain	7.25 ± 0.30	7.11 ± 0.22	13.0 ± 0.47	36.72 ± 0.80

indicative of an increase in JH level. Indeed, 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 are substantially higher than in mature (5–6-days-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, 2003a). 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., 2003a). Considering all the above, we have infered (Gruntenko et al., 2003a) that (i) JH synthesis and degradation are under a common control system in the adult females of *Drosophila*, and (ii) 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)).

In *hs* adult females the response of the JH metabolic system to heat stress is inhibited, but it does not interfere with the response of the 20E system. Besides, *hs* mutants have a significantly higher level of 20E and lower JH degradation level than *wt* flies under normal conditions (Table 12.1).

Based on the hypothesis of Soller et al. (1999) about the importance of the gonadotropin balance in the control of *Drosophila* oogenesis we could expect changes in the progress of oogenesis in mutant females under normal conditions. One would also predict changes in oogenesis in *wt* and *hs* females under heat stress conditions.

Figure 12.1a shows the stage distribution of egg chambers in young *wt* and *hs* females under normal and heat stress (38°C) conditions (Gruntenko et al., 2003b). Under normal conditions *hs* mutant females have a higher proportion of early vitellogenic oocytes (stages 8–9) than *wt* females. The proportion of oocytes in the late stages of development (11–14) is significantly higher in *wt* females. Degenerating stage 8–10 egg chambers are observed in *hs* mutant ovaries under normal conditions. These results suggest that in *hs* mutant flies the transition of oocytes through stage 10 is delayed, as evidenced by the decreased proportion of late stages (11–14)

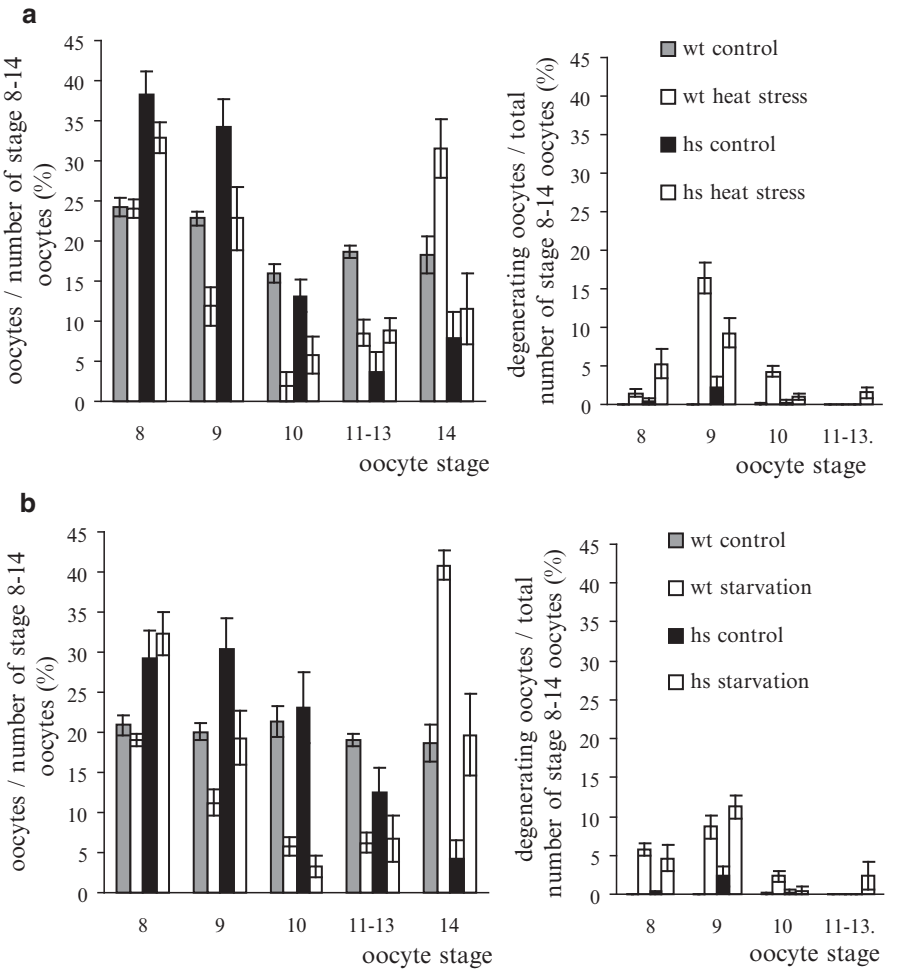

Fig. 12.1 Effects of (**a**) heat (38°C, 4 h) and (**b**) nutrition (flies kept on pure sugar diet during 24 h) stresses on oogenesis in 3-day-old *D. virilis* wild type (*wt*) and *hs* mutant females. Oocyte stages were determined for 7–12 pairs of ovaries after Hoechst staining. Left diagrams show the distribution of egg chambers and right diagrams, the distribution of degenerating egg chambers under normal conditions and 12 h after stresses. Means ± SE

and the increased proportion of early stages (8–9). Together with our data showing the increased 20E level in *hs* mutant flies (Table 12.1), the results are consistent with the model of Soller et al. (1999) suggesting that an elevation of 20E levels in females slows down development to stage 10. Partial degeneration of stages 8–9 oocytes in *hs* mutant females also fits well with the conclusion of Soller et al. (1999) that elevated titres of 20E lead to the resorption of early vitellogenic oocytes.

Figure 12.1a also presents the data on the effect of heat stress on the course of oogenesis in *wt* and *hs* females. In *wt* females there are fewer normal oocytes at

stages 9–13; many egg chambers degenerate during stages 8–10, and mature eggs (stage 14) accumulate. The decreased percentage of early vitellogenic oocytes is caused by the increased 20E titre induced by the heat stress (Table 12.1). One can also see a delay in oocyte transition to stage 10, similar to that observed in the *hs* mutant under normal conditions. This developmental delay is suggested by an increase of the total number (normal and degenerating) of egg chambers at stage 9 and a decrease of the numbers at stages 10–13 as compared to the control. The accumulation of mature eggs in *wt* females exposed to heat stress agrees with our data showing the cessation of oviposition after heat stress, probably resulting from a decrease in JH degradation (an increase in JH titre) (Rauschenbach et al., 1996).

Following heat stress *hs* mutant females also have a reduced proportion of early vitellogenic oocytes, stages 9–10 and an increase in the proportion of degenerating egg chambers at stages 8–9. However, unlike *wt*, *hs* mutant females show neither an accumulation of mature eggs (Fig. 12.1a) nor a cessation of oviposition (Rauschenbach et al., 1996) in response to heat stress. Thus, *hs* mutant females have an ecdysteroid system which responds to heat stress, while their JH system does not; in parallel they have alterations in oogenesis after heat stress at early stages of oocyte development and do not show them at late stages. We have supposed that ecdysteroids make a bigger contribution to the control of early stages of vitellogenesis under stress conditions, while JH is more important for the completion of egg maturation.

To verify this supposition, we studied JH degradation and oogenesis in *hs* and *wt* females following a different kind of stress – namely starvation. We have found that starvation is accompanied by a decrease in JH degradation levels (an increase in JH levels) in females of both *wt* and mutant *hs* strains (Table 12.2).

If the accumulation of mature eggs under stress is due to a JH titre increase (a decrease of the hormone degradation) we could expect that the late stages of oogenesis in *hs* females, which do not change under heat stress (Fig. 12.1a), would change following starvation.

Figure 12.1b shows the stage distribution of egg chambers in young *wt* and *hs* females under starvation (Rauschenbach et al., 2004a). The pattern of stage distribution in the *wt* strain following starvation is similar to that observed following heat stress, i.e. the changes in the progress of oogenesis are similar with different types of stress. The distribution of stages in *hs* mutant females following starvation is similar to that observed in *wt* females. It is important to note that under nutritional stress there is a significant accumulation of mature eggs in *hs* females. The accumulation of mature eggs in *wt* and *hs* females exposed to nutrition stress agrees with our data showing an oviposition arrest under starvation in both (Rauschenbach et al., 2004a).

Table 12.2 Effect of nutrition stress (starvation for 6 h) on JH degradation in young (3-day-old) *D. virilis* females of *wt* and *hs* strains (Rauschenbach et al., 2004a)

	JH degradation (pmol/min/fly)	
	Control	Starvation
wt strain	13.1 ± 0.45	9.31 ± 0.35
hs strain	11.5 ± 0.47	7.6 ± 0.48

Based on the above, we have inferred that (i) oocyte accumulation at stage 14 and an oviposition arrest in females under stress occurred due to the increasing JH titre; (ii) oocyte degradation at stages 8–10 occurred due to the increasing 20E titre (Rauschenbach et al., 2004a).

We have confirmed this inference when studied the effects of exogenous JH and 20E on oviposition and fertility in *wt* females of *D. virilis*:

1. JH treatment (applying 2 μg of JH-III) of *wt* females resulted in an oviposition arrest for 1 day (Rauschenbach et al., 2004a) similar to that observed under heat stress and starvation (Rauschenbach et al., 1996, 2004a). However, unlike heat stress and starvation, it did not cause a prolonged fertility decrease when oviposition renewed (Rauschenbach et al., 2004a).
2. Different from the JH treatment, 20E treatment (24 h feeding by the hormone) of *wt* females did not cause an oviposition arrest. At the same time, the exogenous 20E caused a prolonged decrease of fertility: the fecundity of the 20E-treated flies was 63–74% from the control level for 4 days (Gruntenko et al., 2005a).

12.3 The Mechanisms of Maintenance of JH and 20E Balance in *Drosophila* Females

It has been demonstrated *in vitro* that biogenic amines may have a regulatory action on JH metabolism in bees, locusts, crickets, beetles and cockroaches (Lafon-Cazal and Baehr, 1988; Thompson et al., 1990; Woodring and Hoffmann, 1994; Kaatz et al., 1994; Rachinsky, 1994; Granger et al., 1996; Hirashima et al., 1999b). The effect of 20E on the metabolism of biogenic amines has also been shown in insect (Hiruma et al., 1985; Hiruma and Riddiford, 1990; Hirashima et al., 1999a; Ferdig et al., 2000; Lehman et al., 2000; Mesce, 2002; Zufelato et al., 2004). However, in the available literature we found no researches into the effects of JH on biogenic amines and the effects of biogenic amines on 20E levels in insects.

To study *in vivo* the interplay between biogenic amines, dopamine (DA) and octopamine (OA), and gonadotropins, JH and 20E, in *Drosophila* we estimated JH, 20E, OA and DA levels (i) in *D. melanogaster* females carrying mutations that change drastically OA, DA, JH or 20E levels, and (ii) in wild type females of *D. virilis* and *D. melanogaster* treated with exogenous OA, DA, JH or 20E.

12.3.1 The Interplay Between Biogenic Amines and JH in Drosophila In Vivo

To determine whether biogenic amines affect JH metabolism in *Drosophila in vivo*, we studied levels of hormone degradation in octopamineless females (strain $T\beta h^{nM18}$ of *D. melanogaster* carries a null mutation of the gene *tyramine-β-hydroxylase*, OA biosynthesis enzyme (Monastirioti et al., 1996)) and in females with a twofold increase of the DA content (*D. melanogaster* strains *scarlet ebony* (*ste*) and *ebony*

carry mutation which drastically decrease activity of the enzyme converting DA into *N*-β-alanyldopamine (Perez et al., 1997)). It has been found that both young and mature octopamineless females have JH degradation levels much higher than those in females of the precursor strain (*p845*) from which the octopamineless strain was derived by *P*-element transposition (Monastirioti et al., 1996) and in wild type flies (*Canton S*). At the same time, the young females with a twofold increase of the DA content have considerably lower JH degradation levels and the mature flies have higher its levels compared to wild type (Gruntenko et al., 2000; Rauschenbach et al., 2001; Gruntenko and Rauschenbach, 2004). It should be noted that these changes in JH metabolism are most likely to be due to the mutations changing OA and DA levels rather than to other genes. This is indicated, first, by the difference in JH degradation levels between the strain $T\beta h^{nM18}$ and its precursor strain, *p845*, and second, by the similar levels of hormone degradation in females of the strains *ste* and *ebony* which carry, compared to different genetic backgrounds, a mutation that results in doubling of the DA content.

Based on the above, we suggested that the pattern of the regulation of JH metabolism by the biogenic amines in *Drosophila* includes OA inhibiting JH degradation and stimulating its synthesis (see Section 12.2) both in young and mature *Drosophila* females; DA inhibits hormone degradation and stimulates its synthesis in the young females, and in contrast, it stimulates JH degradation and inhibits its synthesis in mature females (Gruntenko and Rauschenbach, 2004).

To verify this suggestion we studied the effects of DA and OA treatment (feeding flies with the amines) on JH degradation levels in *Drosophila* wild type flies (Gruntenko et al., 2005a, 2007).

Figure 12.2a presents the results of measurements of the JH-hydrolyzing activity in young (2-day-old) and sexually mature (6-day-old) *wt* females, both OA treated and control. The increase in OA content leads in both young and mature females to a decrease of JH degradation (an increase of JH level, see Section 12.2). We obtained similar data from a study of the effect of exogenous OA on JH metabolism in

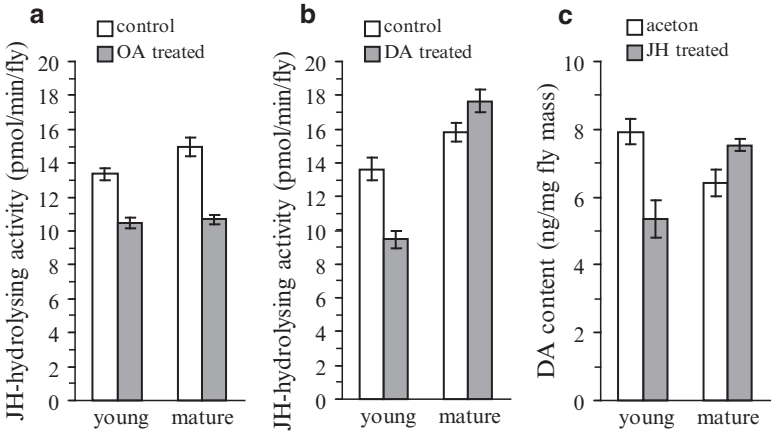

Fig. 12.2 Effects of OA (**a**) and DA (**b**) feeding on JH degradation, and effect of JH application on DA content (**c**) in young and mature females of *D. virilis* wild type. Means ± SE

D. melanogaster (Gruntenko et al., 2007). Note, that the increase in OA content had no effect on JH degradation levels in *Drosophila* males (Gruntenko et al., 2007).

The effect of exogenous DA on JH-hydrolyzing activity in young (2-day-old) and mature (7-day-old) *wt* females is shown in Fig. 12.2b. The increase in DA content leads in young females to a sharp decrease of JH degradation (an increase of JH level) and in the mature females to its increase (decrease of JH level). Ontogenetic differences in the control of JH metabolism by DA has also been established for larvae of *Manduca sexta*: DA stimulates hormone biosynthesis in the corpora allata in the first 2 days of the last larval stage but inhibits the corpora allata on days 3–6, at the beginning of the prepupal stage (Granger et al., 1996). It should be emphasized, that the increase in DA content, like in OA, had no effect on JH degradation levels in *wt* males (Gruntenko et al., 2005a). We have also shown that threefold decrease of the DA content after 3-iodo-tyrosine (an inhibitor of tyrosine hydroxylase) treatment results in a drop of JH degradation (an increase of JH level) in the mature *wt* females (Gruntenko et al., 2005a).

As mentioned above, the *apterous*^{56f} mutation leads to dramatically decreased JH levels in *D. melanogaster* (Altaratz et al., 1991; Gruntenko et al., 2003a). We have found that young *ap*^{56f} females have DA levels twice as high as *Canton S* and *Oregon R* females (Gruntenko et al., 2003a). Although the *ap* gene encodes one of the LIM homeodomain transcription factors which play key roles in a variety of developmental processes (Hobert and Westphal, 2000 for review) our data suggested it was not directly involved in the regulation of DA metabolism. Really, DA content in males of the *ap*^{56f} strain did not differ from the wild type (Gruntenko et al., 2003a). We presumed that the increased DA level in *ap*^{56f} females could be explained by a compensatory response to a reduced JH titre: DA levels rise in young *ap*^{56f} females at the beginning of the oogenesis in order to increase JH titre by stimulation of its synthesis and suppression of its degradation (see above). Support for this hypothesis was provided by our data on JH treatment of young *ap*^{56f} females: the increase of JH level resulted in a decline in DA content making it closer to wild type level (Gruntenko et al., 2003a). Based on these data, we suggested that there is a feedback loop in the regulation of JH metabolism by DA (Rauschenbach et al., 2004b).

The suggestion was confirmed when we studied the effects of exogenous JH on DA contents in young and mature wild type females of *D. virilis* (Rauschenbach et al., 2004b). Figure 12.2c depicts the results of measurements of the DA contents in young (2-day-old) and sexually mature (7-day-old) *wt* females after the application of JH-III dissolved in acetone and in the control treated with acetone. The increase in JH levels leads to the decrease in DA content in young females and to the rise in DA in mature females.

12.3.2 The Interplay Between JH and 20E in Drosophila In Vivo

To find out whether an experimental increase in 20E levels has any effect on JH metabolism in *D. virilis*, the flies were kept for 8 days on a nutrient medium with 20E.

Levels of JH degradation were measured in 20E-treated and control young (2-day-old) and mature (7-day-old) *wt* females. The results are shown in Fig. 12.3a, b. 20E treatment leads to a considerable decrease of JH degradation (an increase of JH level) in young females (Fig. 12.3a). (Note, that this decrease is dose dependent (Gruntenko et al., 2005a).) In the mature females (Fig. 12.3b) too an increase in the 20E level results in a decrease of JH degradation (an increase of JH level). This testifies to the existence of a mutual control of JH and 20E in *Drosophila*: not only does JH stimulate ecdysteroid production (Richard et al., 1998) and increase 20E levels *in vivo* (Fig. 12.3c), but also 20E, in turn, is capable of regulating JH levels.

Another proof of the inference that 20E affects JH metabolism are our results of JH degradation measurement in the *ecdysoneless*^{1} (*ecd*^{1}) mutant of *D. melanogaster*: in 1- and 5-day-old *ecd*^{1} females maintained at 29°C (shifting newly emerged *ecd*^{1} adults to 29°C results in drastically reduced 20E titres (Garren et al., 1977)) JH degradation is significantly higher (JH level lower) as compared to that

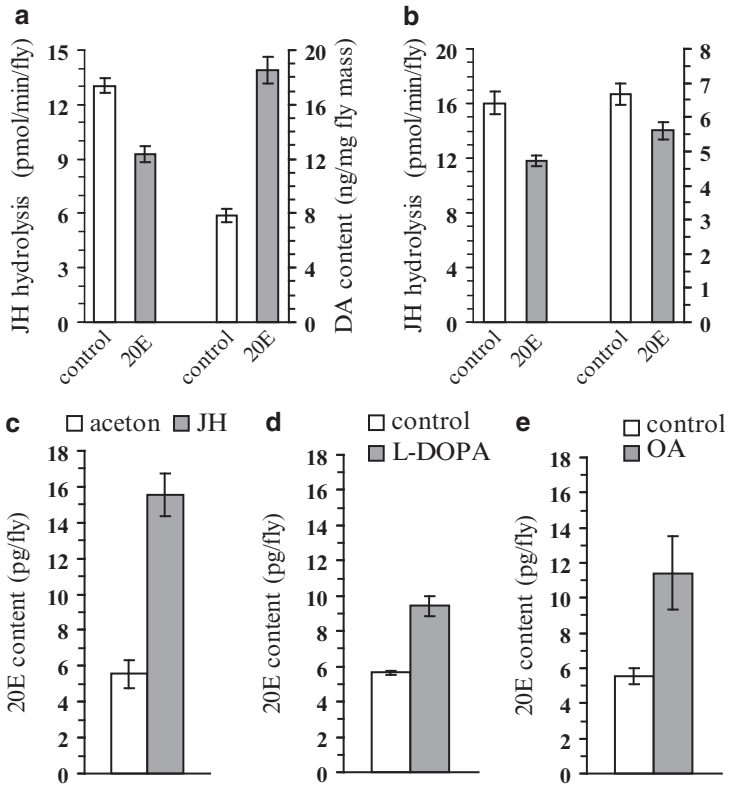

Fig. 12.3 Effects of 20E feeding (**a**, **b**) on JH degradation and DA content in young (**a**) and mature (**b**) *wt* females of *D. virilis*. (**c**) Effect of application of JH-III dissolved in acetone on 20E content in young *wt* females (controls were treated with acetone). (**d**, **e**) Effects of L-DOPA (**d**) and OA (**e**) feeding on 20E content in young *wt* females. Means ± SE

of *ecd*^{1} females kept at the temperature 19°C at which they have the normal 20E level (Karpova et al., 2005).

A question arose whether a change in the 20E level affects directly JH metabolism or is it mediated through the DA system, since we have shown that JH metabolism under normal conditions is regulated by DA (DA inhibits JH degradation in the young females and stimulates it in the mature ones (see above)). The data in Fig. 12.3a show a steep increase of the DA level in young *wt* females upon 20E treatment. This increase is dose dependent (Gruntenko et al., 2005a). However, the DA content in the mature females, unlike that in the young ones, decreases upon 20E treatment (Fig. 12.3b). The influence of 20E on DA levels in *Drosophila* is also confirmed by our data regarding the significant decrease of the DA level in the young and increase in the mature females of the strain *ecd*^{1} kept at 29°C (Karpova et al., 2005).

Taking the above into consideration, it is reasonable to infer that the effect of 20E on JH metabolism in *D. virilis* is mediated through the DA metabolic system. Indeed, (i) an increase in the DA content in the young females after 20E treatment and its decrease in the mature ones should result in a decrease of the JH-hydrolyzing activity (an increase in JH level) in both; (ii) if 20E had a direct effect on JH metabolism, an increased level of the latter in 20E-treated females should lead to a decrease in DA content in young females (see Section 12.3.1) and its increase in the mature ones, and we observe the reverse (Fig. 12.3a, b).

To elucidate the interplay between 20E and biogenic amines, OA and DA, we measured 20E contents in 2-day-old *wt* females of *D. virilis* fed with OA and with DA precursor, L-dihydroxyphenylalanine (L-DOPA). The females fed with L-DOPA had a much higher DA content (greater by the factor of 2.5 (Rauschenbach et al., 2007)).

As mentioned above, an increase in 20E level leads in young *wt D. virilis* females to an increase in DA content. 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 females should result in a decrease in 20E level. Data presented in Fig. 12.3d indicate that this is not the case: feeding the flies with L-DOPA results in the increase of 20E level. 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. 12.2b) should lead to a rise of 20E because JH activates ecdysone synthesis in ovaries of young females (Postlethwait and Shirk, 1981; Kelley, 1994; Richard et al., 1998). Data in Fig. 12.3c correlate with this: in JH-treated *wt* females the 20E level is dramatically increased. Thus we infer that DA has an effect on 20E metabolism, but this effect is indirect and mediated through the JH metabolic system.

The effects of a rise in OA content on 20E level in *wt* females of *D. virilis* are shown in Fig. 12.3e. The increase in OA content in 2-day-old *wt* females leads to the increase of 20E content. The elevation of 20E titre could be due to the OA-induced increase in the JH titre. Indeed, the increase in OA content leads in both young and mature females to a decrease of JH degradation (an increase of JH level, Fig. 12.2a). As mentioned above (see Section 12.3.1), we have also shown that both young and mature octopamineless females of *D. melanogaster* strain $T\beta h^{nM18}$ have JH degradation levels much higher (JH levels much lower) than those in its precursor strain,

p845, flies (Gruntenko et al., 2000). If OA, like DA, regulates 20E through JH metabolic system, one could expect octopamineless females to have 20E level lower than in wild type. Indeed, we have found a considerable decrease in 20E content in $T\beta H^{NM18}$ females (Rauschenbach et al., 2007). It cannot be ruled out, however, that besides the regulation of 20E titre mediated via a JH system OA may also regulate 20E titre directly. This possibility is supported by Hirashima et al. (1999a) who show that exogenous OA affects *in vitro* ecdysteroid synthesis by the prothoracic gland of *Bombyx mori* larvae.

12.3.3 Mechanism of the Reciprocal Regulation of Gonadotropins and Biogenic Amines in Drosophila

Taking all above into consideration, we inferred (Rauschenbach et al., 2007) that a mechanism of the reciprocal regulation of biogenic amines and gonadotropins which maintains the balance of JH and 20E in the case when either of the hormone levels is changed exists in *Drosophila* (Fig. 12.4):

(i) DA increases JH levels (inhibiting JH degradation and apparently stimulating synthesis) in young females and decreases it by stimulating degradation and inhibiting synthesis in sexually mature flies (Gruntenko et al., 2000, 2005b; Gruntenko and Rauschenbach, 2004; see Fig. 12.2).
(ii) There is a feedback loop as part of this regulation – a rise in JH levels leads to the decrease in DA content in young females and to the rise in DA in mature females (Gruntenko et al., 2003b; Rauschenbach et al., 2004b; see Fig. 12.2).

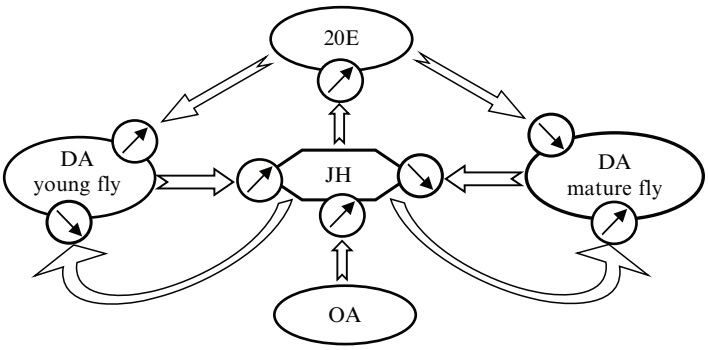

Fig. 12.4 Scheme of the reciprocal regulation of gonadotropins (JH and 20E) and biogenic amines (DA and OA) in *Drosophila*. Arrows in the circles point out effect directions: upward – increasing, down – decreasing

(iii) OA leads to a rise in JH levels by inhibiting JH degradation and apparently stimulating its synthesis in young and mature females (Gruntenko et al., 2000, 2007; Rauschenbach et al., 2007; see Fig. 12.2).
(iv) 20E regulates JH indirectly via DA metabolic pathways – a rise in 20E level increases DA content in young females and decreases it in mature females, thus leading to a decrease of JH degradation and hence a rise in the hormone levels in both (Gruntenko et al., 2005a; see Fig. 12.3).
(v) JH regulates 20E metabolism: a rise in JH titre increases the 20E level in young females (Rauschenbach et al., 2007; see Fig. 12.3).
(vi) DA and OA regulate 20E metabolism via JH metabolic pathways – a rise in DA and OA contents increases JH level in young females, thus leading to a rise in the 20E titre (Gruntenko et al., 2007; Rauschenbach et al., 2007; see Fig. 12.3).

A natural question to ask is how this mechanism works? We suggest the following (Fig. 12.5a, b).

I. If 20E level rises (Fig. 12.5a):

In young females:

1. 20E causes an increase in DA level.
2. The increase of DA level results in a rise in JH titre.

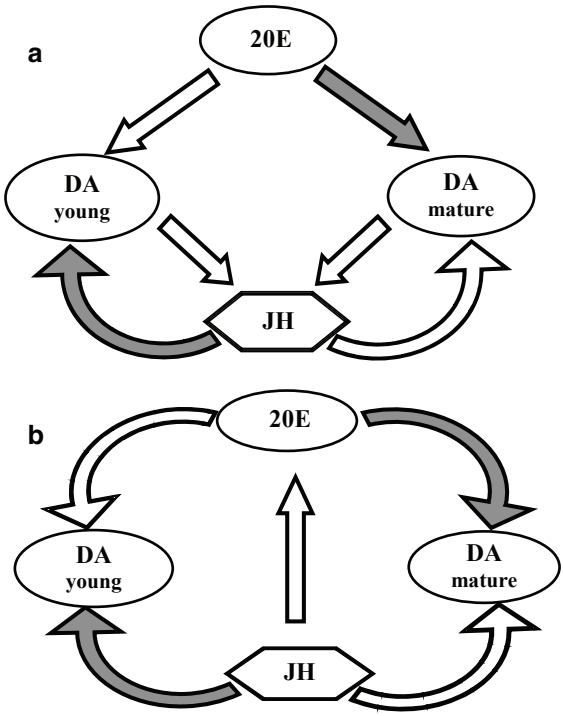

Fig. 12.5 Scheme of functioning of the mechanism maintaining the balance of JH and 20E when either of the hormone levels is changed. (**a**) The case when 20E level rises, (**b**) the case when JH level rises. Closed arrows point out a decreasing effect, open arrows point out an increasing effect

3. The rise in JH titre elicits a decrease of DA content, DA level stabilizes, and the gonadotropin balance sets in at a new level.

In mature females:

1. 20E causes a decrease in DA level.
2. The decrease of DA level results in a rise in JH titre.
3. The rise in JH titre elicits an increase of DA content, its level stabilizes, and the gonadotropin balance sets in at a new level.

II. If JH level rises (Fig. 12.5b):

In young females:

1. JH causes an increase of 20E titre and a decrease of DA content.
2. The increase of 20E titre results in a rise in DA content, its level restores, and the balance of JH and 20E sets in at a new level.

In mature females:

1. JH causes an increase in both 20E titre and DA content.
2. The increase in 20E titre results in a drop of DA content, its level stabilizes, and the gonadotropin balance sets in at a new level.

12.4 Conclusions

The above allows us to conclude:

1. Stress leads to dramatic changes in the reproductive function of *Drosophila* females, the reason being imbalance of gonadotropins which occurs owing to non-simultaneous response of their systems of metabolism to stressor. Indeed, ecdysteroid system responds to 60 min of stress exposure (38°C) with an increase in ecdysone (E) and 20-hydroxyecdysone (20E) levels (Hirashima et al., 2000); JH metabolic system responds to stress with a decrease in JH degradation (increase in JH titre) beginning after 120 min of stress exposure (Rauschenbach et al., 1995, 1996; Gruntenko et al., 2003b).
2. A rise in JH titre under stress is adaptive because it results in mature eggs accumulation and oviposition arrest, allowing the population to "wait" until the end of unfavourable conditions without a considerable decrease in potential numbers; a rise in 20E titre is adaptive under the conditions of overpopulation or reduced food supply because it leads to resorption of a part of egg chambers and a decrease of fecundity.
3. The mechanism of a reciprocal regulation of biogenic amines and gonadotropins which maintains the balance of JH and 20E in the case when either of the hormone levels is changed exists in *Drosophila*. The existence of such mechanism is also adaptive because it promotes restoration of the normal levels of reproduction after stress exposure.

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