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Juvenile hormone, 20-hydroxyecdysone and dopamine interaction in *Drosophila virilis* reproduction under normal and nutritional stress conditions

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Abstract

To elucidate the role of the juvenile hormone (JH) in the control of *Drosophila* reproduction under stress, JH degradation, dopamine (DA) content and reproduction were studied upon 20E treatment in *Drosophila virilis* females of wild type (*wt*) and a mutant, with increased 20E level and decreased fertility, under normal and nutritional stress conditions. 20E treatment of *wt* flies for 7 days results in an increase of DA content in young females, but a decrease in mature females, a decrease of JH degradation in both young and mature females, an 1-day delay in onset of oviposition and a decrease of fecundity to the level typical of mutant flies. One day of 20E treatment in 7-day-old fed and starved flies results in a small decrease of JH degradation in the fed females and a great decrease in the starved ones. Fecundity decreases in the fed flies to the levels of the starved untreated flies in both *wt* and mutant strains. An oviposition arrest is observed in the treated and the untreated starved, but not in the treated fed, females of both strains. The data obtained suggest ecdysone control of JH metabolism mediated via DA.

Keywords: Juvenile hormone; 20-hydroxyecdysone; Dopamine; Fertility; *Drosophila virilis*; Starvation

1. Introduction

Ecdysteroids and the juvenile hormone (JH) are known to play a gonadotropic role in insect reproduction. It has long been postulated that vitellogenesis, the process of yolk protein (YP) synthesis and oocyte uptake, is regulated both by JH (produced in the *corpus allatum*) and by ecdysteroids that are synthesized by the ovary follicular cells and other tissues (Koeppe et al., 1985; Bownes, 1986, 1989). However, recently, Richard et al. (1998, 2001) have proposed a hypothesis that in *Drosophila* JH initiates only early stages of vitellogenesis in the fat body and in the ovary follicular cells as well as ecdysteroid production in the ovary, while 20-hydroxyecdysone (20E) plays the 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. On the other hand, Soller et al. (1999), based on the results of experiments on the effect of exogenous JH and 20E treatment on *Drosophila 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 develop-

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ment. The authors also assume 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 evidence in favor of the latter hypothesis, having studied the effects of shifting the balance between 20E and JH on the process of oogenesis in *Drosophila virilis*: changes in the endogenous levels of 20E and JH as a result of heat or nutritional stress or as a result of a mutation led to serious disturbances in oogenesis (Gruntenko et al., 2003a; Rauschenbach et al., 2004a). We also found that in females of *D. virilis* strain 147 (called *heat stress* (*hs*) mutant), changes are observed under *hs* in early vitellogenic stages (degradation of some egg chambers at stages 8–9 and a delay of oocyte transition through stage 10) but there is no effect on late stages (i.e. there is no accumulation of stage 14 oocytes typical of wild type (*wt*) females) (Gruntenko et al., 2003a). In *hs* females, the response of the JH metabolic system to *hs* is inhibited (Rauschenbach et al., 1995, 1996), but does not interfere with the response of the 20E system (Gruntenko et al., 2003a). However, in females of this strain the JH metabolic system responds to the nutritional stress (the hormone degradation decreases, like in the *wt*), and they accumulate mature oocytes and stop laying eggs when starved (Rauschenbach et al., 2004a). A similar oviposition arrest is observed in *wt* females upon JH application (Rauschenbach et al., 2004a). Based on these data we have concluded that under stress 20E controls the early stages of oogenesis and JH, the late ones and oviposition.

Here, we report data on the effect of an experimental increase of 20E titer on JH metabolism, dopamine (DA) content and fecundity, which support both the hypothesis by Soller et al. (1999) about the importance of the gonadotropins 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.

2. Materials and methods

2.1. Maintenance of stocks

Two lines of *D. virilis* were used: 101, *wt*, and mutant line 147 (*hs* mutant), carrying mutations *brick*, *broken*, and *detached* on chromosome 2 and a temperature-sensitive conditional larval lethal on chromosome 6 (Rauschenbach et al., 1984). Adult *hs* females show increased levels of both 20E and ecdysone and decreased fertility under normal conditions (Rauschenbach et al., 1996; Hirashima et al., 2000).

Flies were maintained on a standard yeast, cornmeal, sugar, nipagin and agar medium at 25 °C. Flies were synchronized at eclosion (flies were collected that eclosed within 3–4 h).

2.2. JH hydrolysis assay

In the present study, we measured JH hydrolysis by the partition assay of Hammock and Sparks (1977), because earlier we showed that in *D. virilis* major JH degradation was carried out by JH-esterase (JHE), and the activity of JH-epoxide hydrolase was low and did not change under stress (unlike that of JHE which decreases steeply under stress conditions of various natures) (Rauschenbach et al., 1995; 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 using) and 12,500 dpm [^{3}H]-JH-III labelled 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

DA content was measured using a slightly modified method of Maickel et al. (1968). There is a difference in the technique for the extraction of biogenic amines from tissue: we used $HClO_4$, whereas Maickel et al. (1968) used acidified butanol for this purpose. However, we obtained evidence that this difference did not affect the results: there was no significant difference whether perchloric acid or acidified butanol was used for extraction, as reported elsewhere (Rauschenbach et al., 1993). Flies were weighed and homogenized on ice in 0.1 M $HClO_4$ (1 fly in 0.12 ml). Sample size varied from 10 to 15 individuals for each group. The homogenates were centrifuged for 10 min at 12,000 rpm and DA content was determined in 0.1 ml of supernatant. The method of Maickel et al. (1968) is based on the specific fluorescence of amines when oxidized by iodine. To 0.1 ml of the supernatant 0.05 ml of 0.4 N HCl, 0.1 ml of EDTA (pH 7.0) (for the fluorofor stabilization), and

0.1 ml of iodine dissolved in alcohol were added successively. The mixture was incubated for 2 min at room temperature, then 0.1 ml of sodium alkaline sulphite was added to interrupt the iodine oxidation of the amines. In 1.5 min 0.1 ml of 10 N acetic acid was added and the tubes were gently shaken. The samples were heated on water bath at 96 °C for 6 min and cooled in ice water right away. One milliliter of the twice-distilled water was added to each sample and the measurements were taken using a Hitachi fluorimeter (the wavelengths were 330 nm for exciting light and 370 nm for emitted light). DA concentrations were calculated by comparing sample values to that of the DA standard.

2.4. Fecundity analysis

Fecundity analysis was as follows: three newly eclosed females and three males were placed into vials (10–12 vials in each control and experimental series), the bottom and walls of which (1 cm high) were covered with filter paper wetted with 0.5 ml of the nutrition medium which contained 0.5% sucrose and 0.2% yeast. In the experimental series, 30 or 60 μg of 20E (Sigma) were added to this solution according to the modified method of Farkaš and Knopp (1977). [In the study of fecundity upon 1-day starvation the nutrition medium contained 0.5% sucrose only; 20E was added to the solution in the experimental series only on that day.] Flies were transferred to vials with fresh nutrition medium daily for 8 or 11 days. Fecundity was determined as the number of eggs laid by a female within 24 h.

2.5. Statistical analysis

Statistical analyses were performed using Student's *t*-test and ANOVA procedures in STATISTICA for Windows, Release 4.5 (@StatSoft Inc., 1993).

3. Results

3.1. Effects of continuous 20E treatment on JH metabolism and DA content in D. virilis wt strain

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 (just starting to oviposit) and mature *wt* females. The results are presented in Figs. 1 and 2.

Fig. 1 shows that 20E treatment leads to dose-dependent decrease of JH degradation in young females. The comparison of control and 20E-treated females by

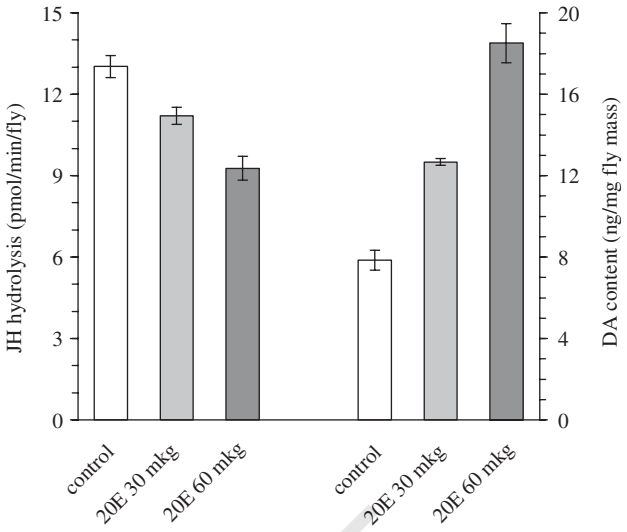

Fig. 1. Effect of 72 h of 20E feeding (30 or 60 μg 20E per vial) on JH degradation and DA content in 3-day-old *wt* females of *D. virilis*. Means ± SE.

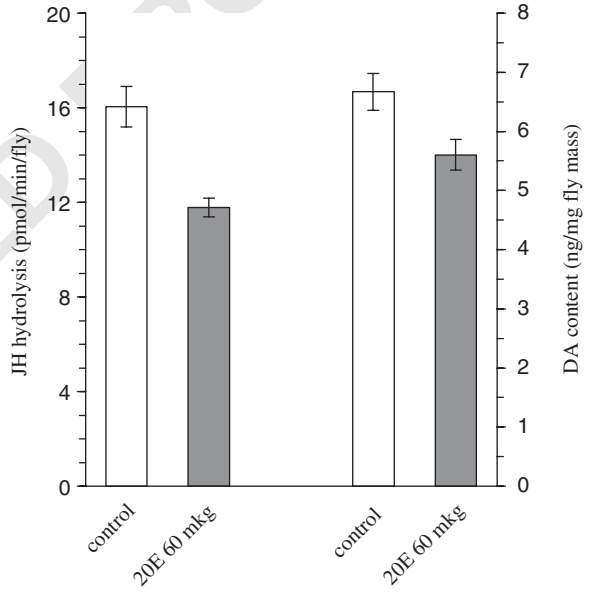

Fig. 2. Effect of 7 days of 20E feeding (60 μg 20E per vial) on JH degradation and DA content in 7-day-old *wt* females of *D. virilis*. Means ± SE.

one-way ANOVA, with 20E treatment dosage as fixed effect, revealed significant effects of 20E concentrations ($p < 0.001$) for JH-hydrolyzing activity.

A question arises as to whether a change in the 20E level directly affects JH metabolism or whether the effect is mediated through the biogenic amine system, since earlier we have shown that JH degradation under normal conditions is regulated by DA (DA inhibits JH degradation in the young females and stimulates it in the

mature ones (Gruntenko et al., 2000; Gruntenko and Rauschenbach, 2004)). The data in Fig. 1 show dose-dependent increase of the DA level in young *wt* females upon 20E treatment. Use of an one-way ANOVA on the data for control and 20E treated females (20E concentrations as fixed effect) revealed that 20E significantly ($p<0.001$) affected DA levels.

Fig. 2 represents changes in the DA content and the levels of JH degradation in 20E-treated and control mature (7-day-old) *wt* females. It is clear that in the mature females too an increase in the 20E level results in a decrease of JH degradation (differences from the control are significant at $p<0.01$). However, the DA content in the mature females, unlike that in the young ones, decreases upon 20E treatment as compared to the control group (differences are significant at $p<0.01$).

3.2. Effect of continuous 20E treatment on the fecundity of D. virilis wt strain

To find out how the changes in the hormonal status of the females affect fecundity upon treatment with 20E, this parameter was measured in the *wt* flies 20E-fed for 8 days (the dose of the hormone—60 μg). These data were compared with those from a control group of *wt* flies and the mutant *hs* flies characterized, as mentioned above, by much higher ecdysteroid levels than *wt* (Hirashima et al., 2000). As one can see from the data on Fig. 3, fecundity of *wt* flies treated with 20E drops sharply, in comparison with the control flies of the same strain, starting on day 5 after eclosion (differences from the control on days 5–8 are significant at $p<0.001$), and starting from day 6 it does not differ from fecundity of *hs* mutants. Noteworthy is a delay in oviposition onset in the 20E-treated *wt* females. It has to do, apparently, with the enhanced, as compared to control, levels of both gonadotropins (a putative increase of JH titer in this experiment will be considered in the Discussion). Earlier, we observed a similar delay after JH treatment of *hs* mutants with the increased endogenous ecdysteroid level (Rauschenbach et al., 2002).

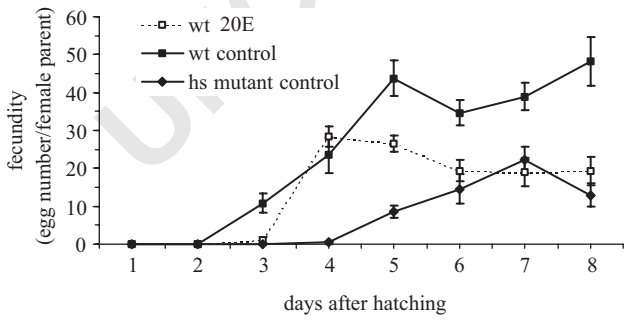

Fig. 3. Effect of 8 days of 20E feeding (60 μg 20E per vial) on fecundity of *wt* flies in comparison with *wt* and *hs* mutant untreated flies. Means ± SE.

3.3. Effect of 1-day 20E treatment and starvation on fecundity of wt and hs strains

Earlier, we showed that JH treatment (applying 2 μg of JH-III) of *wt* females resulted in an oviposition arrest similar to that observed under starvation (Fig. 4B, data reproduced from Rauschenbach et al., 2004a). To test if treatment with 20E can produce the same effect, we evaluated fecundity of the *wt* and *hs* females 20E-fed for 24 h in comparison with that of the yeast-fed and starved (pure sugar-fed) groups (Figs. 4A and 5).

The data in Fig. 4A shows that unlike the JH treatment (Fig. 4B) and starvation, 20E treatment does not cause an oviposition arrest. The exogenous 20E causes a prolonged decrease of fecundity: on the day of treatment (7th day after eclosion), the fecundity of the 20E-treated flies is 71% from the control level (differences are significant at $p<0.05$); on days 9, 10 after eclosion it is 63% and 74%, accordingly (differences are significant at $p<0.01$ for both days), which corresponds to the fecundity levels observed on days 9 and 10 in the starved untreated flies. 20E-treatment of the starved flies, unlike their JH-treatment (Fig. 4B), does not lead to fecundity changes on the next day (day 8 after eclosion for 20E treatment and day 4 for JH treatment), but it decreases fecundity significantly ($p<0.05$) 24 h later as compared to that of the untreated starved flies (Fig. 4A). On day 4 after the treatment (day 11 after eclosion), differences between all groups disappear.

The data in Fig. 5 demonstrate that the pattern of changes in the fecundity levels caused by the exogenous 20E in the flies with an increased, as compared to the *wt*, endogenous 20E level (*hs* mutant) is similar to that found in *wt* flies. However, fecundity of 20E-treated *hs* flies decreases on the day of treatment to level that is lower than that of *wt* flies—it is only 62% from the control level (differences are significant at $p<0.01$).

Noteworthy is a rise in fecundity on day 1, after the end of starvation in females exposed to nutritional stress on day 7 after eclosion (Fig. 4A). When *wt* flies were starved on day 3 after eclosion this effect was not observed (Fig. 4B). We suppose that this happens because in the control females on day 8 after eclosion a fertility drop occurs under normal conditions (which usually takes place in *D. virilis* females 7, 8 or 9 days after eclosion before re-mating), thus making the effect of laying the accumulated eggs more noticeable than during a fertility rise on day 4 after eclosion (Fig. 4B). Earlier, we showed that the eggs accumulated during the oviposition arrest in the females starved on day 3 after eclosion were laid during the first 3 h after the end of starvation, and then a decrease of fertility started which disguised this peak when the total number of eggs laid within 24 h was counted (Rauschenbach et al., 2004a).

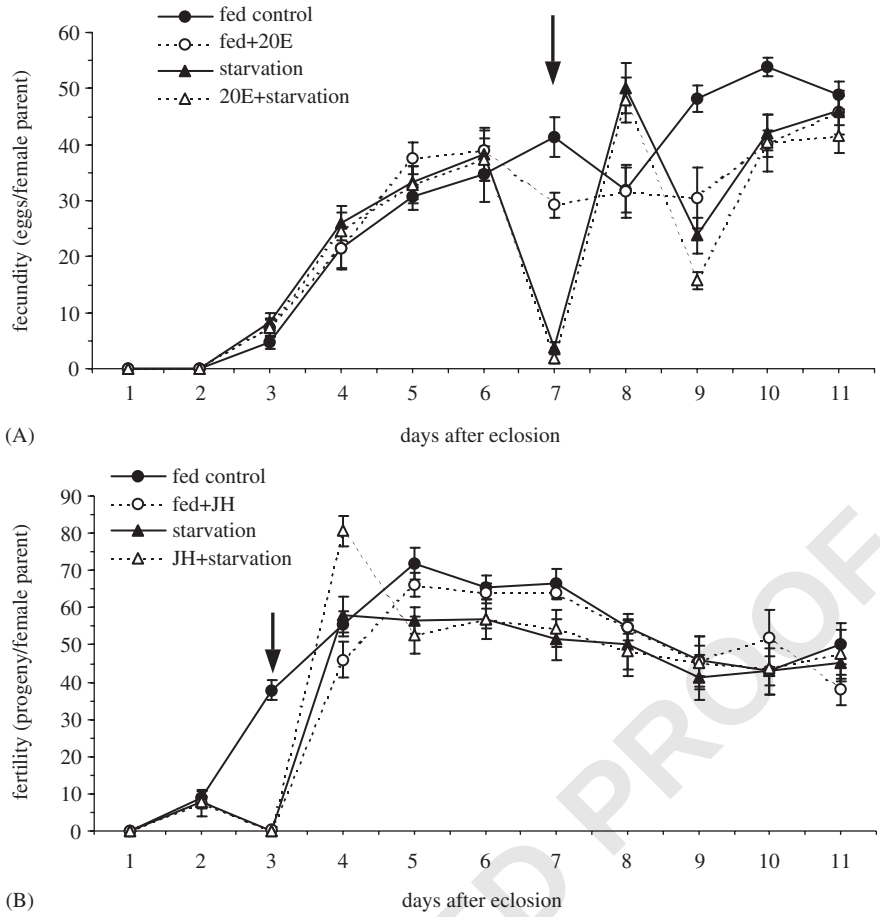

Fig. 4. Effect of 24 h of 20E feeding (A, 60 μg of 20E per vial) and of JH application (B, 2 μg of JH per female, reproduced from Rauschenbach et al., 2004a) on reproduction of fed (yeasts and sugar medium) and starved (pure sugar medium) for 24 h *wt* flies of *D. virilis*; untreated fed and starved flies were used as control. The day of treatment is shown by arrow. Means ± SE.

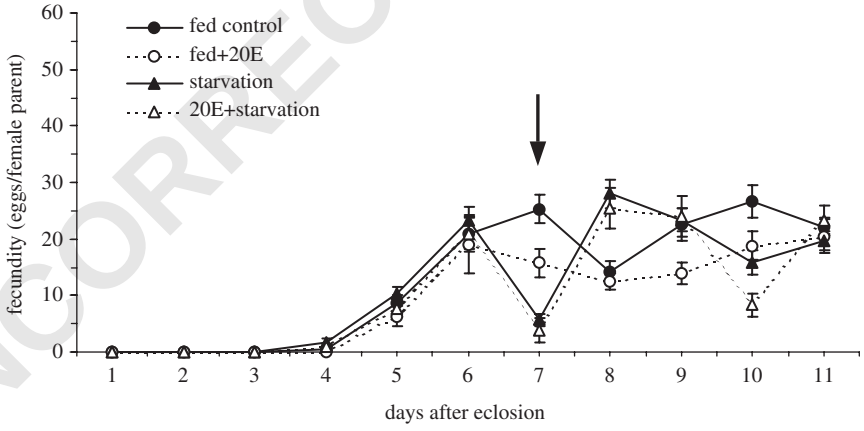

Fig. 5. Effect of 24 h of 20E feeding (60 μg of 20E per vial) on fecundity of fed (yeasts and sugar medium) and starved (pure sugar medium) for 24 h *hs* mutants of *D. virilis*; untreated fed and starved flies were used as control. The day of treatment is shown by arrow. Means ± SE.

3.4. Effect of 1-day 20E treatment and starvation on JH metabolism in wt females

Fig. 6 presents JH degradation levels measured 18 h after the start of treatment in *wt* starved females (exposed to 24 h of nutritional stress), fed females treated with 20E, starved females treated with 20E, and fed controls. One can see that 20E feeding for 18 h decreases JH degradation level by 20% (differences from the control group are on the margin of significance); starvation decreases it by 40%; starvation and 20E treatment, by 45%. A comparison of these results with

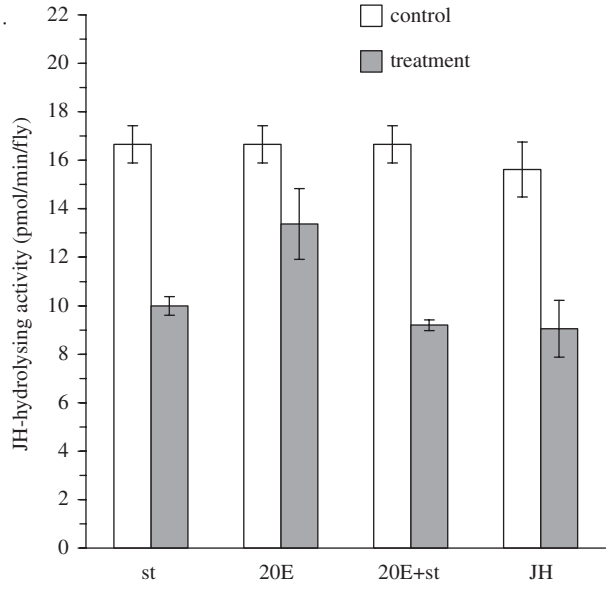

Fig. 6. Effect of 20E feeding, starvation and JH application (reproduced from Rauschenbach et al., 2004a) on JH degradation in *wt* females of *D. virilis*. Means ± SE. st—18 h of starvation (pure sugar medium); 20E—18 h of 20E feeding (yeasts and sugar medium with 60 μg 20E per vial); 20E + st—18 h of starvation together with 20E feeding; JH—18 h after JH application (2 μg of JH per female).

the data on JH degradation after the treatment with exogenous JH (data reproduced from Rauschenbach et al., 2004a) shows that JH treatment decreases JH degradation to the same extent (42%) as starvation.

4. Discussion

Hormonal control of the vitellogenin production in the ovary and in the fat body of *D. melanogaster* was studied in detail by Postlethwait et al. (Postlethwait and Handler, 1978; Jowett and Postlethwait, 1981; Postlethwait and Parker, 1987; Postlethwait and Shirk, 1981). They proposed the following mechanism of the control: JH from the *corpora allata* is transfered to the ovary where it stimulates synthesis of YPs in the follicular cells and their further uptake by the oocytes. Simultaneously, JH induces ecdysone-secreting ovary cells to produce ecdysteroids which initiate YP synthesis in the fat body. The latter are secreted to the hemolymph and taken up later by the oocytes. JH stimulation of ecdysteroid synthesis in the ovary of *D. melanogaster* was confirmed in the study by Richard et al. (1998) who showed an increase of ecdysteroid biosynthesis in the case when the ovaries of young females (within 18 h after eclosion) were incubated with JHB_3. At the same time, in this paper, the authors also showed that a sharply reduced JH synthesis in the mutant *apterous*^{56f} (*ap*^{56f}) of *D. melanogaster* (Altaratz et al., 1991) did not result in a decrease of 20E titer. On the contrary, *ap*^{56f} females are characterized by steeply increased levels of production of ecdysteroids in the ovary for the first 3 days after eclosion (Richard et al., 1998).

We assume that this testifies to the existence of a mutual control of JH and 20E in *Drosophila*: not only does JH stimulate ecdysteroid production, but also 20E, in turn, is capable of regulating JH levels. In this case, an increased ecdysteroid level in the *ap*^{56f} mutant may be regarded as a compensatory reaction to the decreased JH titer, aimed at its increase. The data of the present study confirm this assumption: 20E treatment of the *wt D. virilis* females leads to a decrease in JH degradation both in the young (see Fig. 1) and mature (see Fig. 2) females. Furthermore, this decrease is dose-dependent: addition of 30 and 60 μg of 20E to the nutrient medium results in a decrease of JH-hydrolyzing activity by 14% and 29%, correspondingly (see Fig. 1). Another proof of the supposition that 20E affects JH metabolism are our results of JH degradation measurement in the *edysoneless*^{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 ecdysone titers (Garren et al., 1977)), JH degradation is significantly higher as compared to that of *ecd*^{1} females kept at 19 °C (unpublished data).

Earlier, we showed that JH application to *D. virilis* females resulted in a drastic decrease of the hormone degradation (Rauschenbach et al., 2002; see Fig. 6). We assumed this to be a consequence of the common control of JH synthesis and degradation in *Drosophila*. This suggestion was supported by the study of JH metabolism in *ap*^{56f} flies: despite the steeply decreased JH synthesis (Altaratz et al., 1991), *ap*^{56f} females have a sharply increased activity level of the JH-degrading enzymes (Gruntenko et al., 2003b). The existence of a common control of JH production and degradation agrees with the fact that in *wt* adults of *D. melanogaster* regulation of JH synthesis and degradation tend to be opposing. Indeed, both JH titre (Bownes and Rembold, 1987; Sliter et al., 1987) and JH synthesis (Altaratz et al., 1991) in young *wt* (*Canton S*) females were substantially higher than in mature flies; at the same time, JH degradation in young *Canton S* females is significantly lower than in mature females (Gruntenko et al., 2000, 2003b). The notion of a 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 an increase in the activity of JHE, which degrades the hormone.

Thus, a decrease of JH degradation in response to the experimental increase of 20E level (see Figs. 1 and 2) implies, by all appearance, an increase in JH titer in the

wt females of *D. virilis*. This agrees both with the concept of Soller et al. (1999) on the necessity of the proper balance of 20E and JH for the normal progress of oogenesis and with the above suggestion regarding the mutual regulation of the gonadotropins in *Drosophila*.

Noteworthy are the changes in DA content upon 20E treatment found in the present study: an increase of 20E level causes a rise in the DA content in the young females and a decrease in the mature ones (see Figs. 1 and 2). Furthermore, like with JH degradation, these changes are dose dependent (see Fig. 1). 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} *D. melanogaster* (unpublished data).

It is well known that in various insect species, biogenic amines (octopamine and DA) affect JH synthesis and degradation (Thompson et al., 1990; Pastor et al., 1991; Woodring and Hoffmann, 1994; Granger et al., 1996; Hirashima et al., 1999). Having studied JH degradation in *D. melangogaster* females of the octopamineless strain $T\beta h^{nM18}$ and two strains of independent origin carrying the mutation *ebony*, which doubles DA content, we have found that octopamine inhibits JH degradation both in young and mature females, and DA inhibits JH degradation in young but stimulates it in mature *Drosophila* females (Gruntenko et al., 2000; Rauschenbach et al., 1987; Gruntenko and Rauschenbach, 2004). Furthermore, there is a feedback in this regulation: JH application results in a decrease of DA content in the young and its increase in the mature *D. virilis* and *D. melanogaster* females (Gruntenko et al., 2003b; Rauschenbach et al., 2004b). The existence of ontogenetic differences in the control of JH synthesis was demonstrated in females of *Blattella germanica*: DA stimulated JH production on days 1 and 2 of the first ovarian cycle and caused the opposite effect on days 6 and 7 (Pastor et al., 1991).

Taking the above into consideration, it is reasonable to assume that the effect of 20E on JH metabolism in *D. virilis* is mediated through the DA metabolic system. Indeed, an increase in the DA content in the young females after 20E treatment (see Fig. 1) and its decrease in the mature ones (see Fig. 2) should result in a decrease of the JH-hydrolyzing activity. This assumption is also in good accord with the earlier found increased DA content in the young females of the JH-deficient strain ap^{56f} of *D. melanogaster* that decreased upon JH application (Gruntenko et al., 2003b). Based on our hypothesis that JH regulation by 20E is mediated through the DA metabolic system, the increased ecdysteroid level (Richard et al., 1998) in the young ap^{56f} mutant might have been a beginning of a compensatory reaction aimed at an increase in the JH titer via the increase in the DA content.

An important result of the present study is a proof of the conclusion made before that the oviposition arrest under stress is caused by an increase in the JH level, and the further decrease of fertility, by a rise in the 20E titer. Indeed, a rise in the 20E titer does not interrupt oviposition (see Fig. 4A) regardless of the fact that it results in a decrease of JH-hydrolyzing activity (see Fig. 6). Apparently, the decrease in JH degradation (a putative increase in the hormone titer) by 20% observed upon 1-day 20E treatment is not enough to stop oviposition. At the same time, prolonged 20E treatment decreases fecundity of *wt* females (see Fig. 3) to the level typical of the *hs* mutant with the increased endogenous 20E titer (Hirashima et al., 2000), and 1-day 20E treatment decreases fecundity of *wt* and *hs* flies to the level observed in the flies stressed by starvation (see Figs. 4A and 5). The latter is not a surprise, since it was demonstrated that treatment of *D. melanogaster* females with exogenous 20E (Soller et al., 1999) or an increase of the endogenous 20E level in *D. virilis* as a result of stress or a mutation (Gruntenko et al., 2003a; Rauschenbach et al., 2004a) lead to degradation of early vitellogenic oocytes.

It is interesting to note that 20E treatment of *wt* and *hs* females of *D. virilis* simultaneously with nutritional stress leads to a more pronounced fecundity decrease (see Figs. 4A and 5), unlike the JH treatment combined with starvation which results in a fecundity (Rauschenbach et al., 2004a) and a fertility (see Fig. 4B) peak after the end of the oviposition arrest because it ensures, as Soller et al. (1999) showed, protection of a part of early vitellogenic oocytes from 20E-induced resorption.

Thus, the data obtained (1) agree with our supposition about the mutual regulation of the gonadotropins under stress in *Drosophila*, (2) present another proof to the conclusion of Soller et al. (1999) regarding the necessity of the proper balance between JH and 20E for the normal progress of vitellogenesis in *Drosophila* and (3) support the hypothesis of Richard et al. (1998) that 20E might play the main role (including JH titer regulation) in this process.

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