

SHORT TAKE

Impaired ovarian ecdysone synthesis of *Drosophila melanogaster* insulin receptor mutants

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Introduction

Deficient juvenile hormone synthesis is thought to extend adult longevity in insects, including *Drosophila melanogaster*, the Monarch butterfly and several species of Mediterranean grasshopper (Pener, 1972; Herman & Tatar, 2001; Tatar & Yin, 2001). In the monarch and the grasshopper, surgical removal of the adult JH synthetic tissue (corpora allata) mimics adult diapause while it eliminates reproduction and increases longevity. In *D. melanogaster*, adult diapause also is favoured by reduced JH and is associated with suppressed reproduction and slow aging (Tatar *et al.*, 2001a). Aspects of this diapause syndrome are recapitulated in *D. melanogaster* mutant for the insulin-like receptor (*InR*), which are conditionally non-reproductive, long-lived and deficient in JH (Tatar *et al.*, 2001b).

In each system sketched above, JH, a mere sesquiterpenoid, was demonstrated to have a direct effect upon aging because treatment with the hormone restored reproductive activity and normal longevity. Juvenile hormone is but one of the major endocrine factors found in adult insects. Other endocrines include the steroids α -ecdysone and its active form 20-hydroxyecdysone (20-HE). Both JH and 20-HE are best understood as regulators of development, but in the adult they have been studied primarily as they affect oogenesis and vitellogenesis (recent reviews in Kozlova & Thummel, 2000; Truman & Riddiford, 2002). These detailed studies show that JH and ecdysone work in tandem. Indeed, the nuclear hormone receptor for ecdysone, EcR, dimerizes with the nuclear receptor ultraspiral (USP) which has JH as its most likely ligand (Jones & Sharp, 1997; Jones *et al.*, 2002).

Although the potential impact of ecdysone upon aging has yet to be reported, from basic studies of insulin function we predict that ovaries of long-lived *D. melanogaster* mutant for *InR* will produce little ecdysone. In *D. melanogaster*, insulin signal was found to mediate ovarian stem cell proliferation in

response to diet (Drummond-Barbosa & Spradling, 2001), and with fewer eggs, total ecdysone production may be reduced. In another dipteran, the mosquito, ovaries produce ecdysone following a blood meal and this synthesis is insulin dependent; ovaries of sugar-fed females produce ecdysone when stimulated by insulin, and they do so in a PI3-kinase, PKB/AKT, PIP₃-dependent manner (Riehle & Brown, 1999). Through either avenue, hypomorphism of *InR* should affect titres of adult ecdysone.

In the initial course of characterizing the longevity-extending genotypes of *InR* (Tatar *et al.*, 2001b), we investigated the ecdysone production of ovaries from females possessing *InR*⁺, *InR*^{E19} and *InR*^{p5545} alleles. Here we report our preliminary findings. Although limited in scope, the data clearly show that reduced ecdysone synthesis is a phenotype of *InR* mutant alleles. From these simple data we hope to stimulate, perhaps in others, an effort toward detailed investigation of ecdysone action upon aging.

The study

In adult females the active form of ecdysone, 20-HE, is produced by follicle cells of the egg, and to a lesser extent by other tissues (Delbecque *et al.*, 1990). We assessed ovarian synthesis of total ecdysteroids (α -ecdysone and 20-HE) as a function of *InR* genotype: *InR*^{+/+}, *InR*^{+/p5545}, *InR*^{+/E19} which have normal growth and longevity, *InR*^{E19/E19} which has reduced growth but normal life span, and *InR*^{E19/p5545} which is dwarf and long-lived. At the time of these measures, contemporaneous with the work reported in Tatar *et al.* (2001b), the alleles had been back-crossed 10 generations to the wild-type isogenic control (*ri, red, e, InR*⁺). Ovaries in sets of 20 were dissected from virgin females at 6-h intervals within the first 72 h post-eclosion and incubated in buffered Ephrussi–Beadle Ringers media for 5 h (Warren & Mahowald, 1979). In cases where low ecdysteroids were synthesized, 40–80 pairs of ovaries were pooled together for measurement. In general, three to six replicate sets were assessed for each time point and genotype. Ecdysteroids were measured by RIA following Bollenbacher *et al.* (1975); anti-ecdysone antiserum was kindly provided by Gilbert and Bollenbacher (University of North Carolina). The assay was linear in the range of 10–1000 pg using α -ecdysone (Sigma) as standard.

Ecdysteroid synthesis (Fig. 1) was minimal in the ovaries of both dwarf genotypes and could be detected only after 48 h post-eclosion (timing evaluated with *InR*^{p5545/E19}). Ovaries of wild-type and heterozygotes, in contrast, produced up to 90 pg per incubation, and with strong detection as early as 18 h post-eclosion. The age-dependent kinetics of ecdysone synthesis

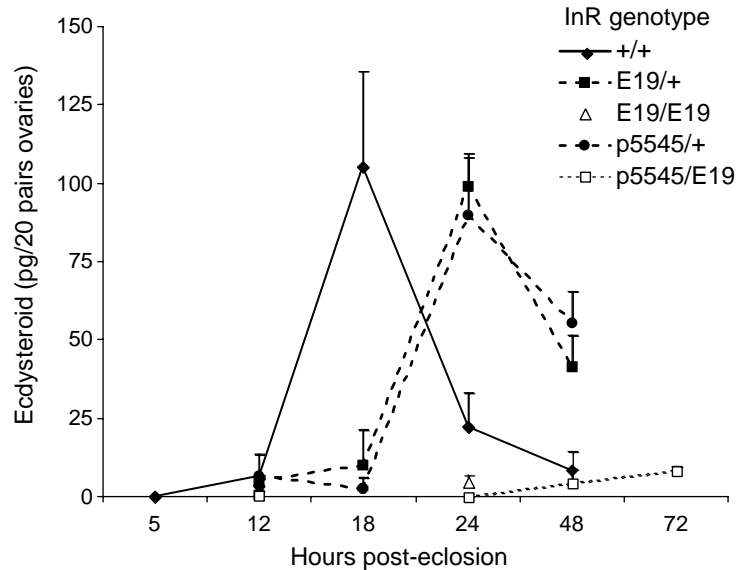
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Fig. 1 Ovarian production of ecdysteroids from virgin *Drosophila melanogaster* females. At each datum, ecdysteroid synthesis was determined relative to α -ecdysone standard (Sigma) after 5 h incubation of at least 20 ovaries. Replicate means with one standard deviation are plotted. Factorial ANOVA was used to assess differences in the cumulative and the time distribution of ecdysteroid produced by *InR*^{+/+} relative to *InR*^{-/+} (*InR*^{E19} and *InR*^{p5545} data combined). The genotypes differed only by 6.84 pg in overall ecdysteroid synthesis ($P = 0.054$); genotype-by-hour interaction was large and significant ($F = 105.0$, $P < 0.0001$).



from ovaries of heterozygotes was significantly delayed relative to the wild-type females, despite the homogeneity of body size and developmental rate among these genotypes (Tatar *et al.*, 2001b).

Concluding comments

Ecdysteroid synthesis is reduced in ovaries of *InR* mutant females. Egg maturation is retarded or almost absent in the dwarf genotypes (Tatar *et al.*, 2001b), perhaps as a direct result of hypomorphic insulin signal within the ovary (Drummond-Barbosa & Spradling, 2001). Few ecdysone-capable follicles are produced. *InR* hypomorphism may also directly impede activation of follicle hormone synthesis (Riehle & Brown, 1999). It would be useful at this stage to challenge the ovaries of dwarf genotypes with insulin in an *in vitro* assay, as was done for mosquito. We attempted such a study, but were unable to affect ecdysone production in any genotype, including controls; further development of the protocol is required.

The consequences of reduced ecdysone for *D. melanogaster* aging are unknown. Strains selected for survival and late-age reproduction had reduced ecdysteroids titres only on the first day post-eclosion (Harshman, 1999). We do not find a one-to-one correspondence between hormone synthesis and longevity among the assessed *InR* genotypes. In particular, *InR*^{E19/E19} had reduced ecdysone (and JH) yet a normal life span (Tatar *et al.*, 2001b). This disparity could arise if some *InR* genotypes in fact slow aging but also incur countervailing age-independent mortality, as seen with some genotypes of *chico* (Tu *et al.*, 2002). Direct manipulation of ecdysone function is required to assess the impact of this steroid upon aging.

The consequences of reduced ecdysone with respect to reproduction are complicated. The matter is important to consider because studies of gonadal ablation with *Caenorhabditis elegans* suggest that gonad-derived signals influence aging.

Ecdysone and a pulse of JH upon eclosion synergistically act to initiate oogenesis (Kelly, 1994). In sexually mature females these hormones are thought to interact through physiological feedback (Soller *et al.*, 1999). The haemolymph titre of ecdysteroids is highest in unmated *D. melanogaster* females (Harshman, 1999); virgins produce a full clutch of eggs, a source of 20-HE, before arresting oogenesis. Hemolymph 20-HE that is unopposed by JH induces resorption of immature eggs. Male-transmitted sex peptides re-stimulate corpora allata JH synthesis and thus release the resorption checkpoint. Newly synthesized ecdysone activates fat body, where yolk protein synthesis is reinitiated. From the details of this example we may anticipate that the relationship between ecdysone and aging also will be complex.

Gonadal signals affecting *C. elegans* life span are Daf-16 dependent and could involve feedback to neuroendocrine cells that express this transcription factor (Arantes-Oliveira *et al.*, 2002). Ecdysone synthesized by follicles of insects could serve this hypothesized function; 20-HE suppresses corpora allata JH synthesis in the cockroach (Rankin & Stay, 1985) although direct suppression is not reported for *D. melanogaster* (Bownes, 1989). Alternatively, reduced ecdysone may alter the overall investment in eggs and thus attenuate one postulated reproductive trade-off with life span. At the same time, ecdysone may have direct effects upon metabolic or stress physiology; notably, JH appears to limit resistance to thermal and oxidative challenge (Salmon *et al.*, 2001; Tatar *et al.*, 2001a). As a ligand to the EcR/USP nuclear hormone complex, there is tremendous combinatorial potential for ecdysone to regulate genes with diverse tissue and physiological specificity.

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References

- Arantes-Oliveira N, Apfeld J, Dillin A, Kenyon C (2002) Regulation of lifespan by germline stem cells in *C. elegans*. *Science* **295**, 502–505.
- Bollenbacher WE, Vedeckis WV, Gilbert LI, O'Conner JD (1975) Ecdysone titers and prothoracic gland activity during the larval-pupal development of *Manduca sexta*. *Dev. Biol.* **44**, 46–53.
- Bownes M (1989) The roles of juvenile hormone, ecdysone and the ovary in the control of *Drosophila* vitellogenesis. *J. Insect. Physiol.* **35**, 409–413.
- Delbecque JP, Weidner K, Hoffmann KH (1990) Alternative sites for ecdysteroid production in insects. *Invertebrate Reprod. Dev.* **18**, 29–42.
- Drummond-Barbosa D, Spradling AC (2001) Stem cell and their progeny respond to nutritional changes during *Drosophila* oogenesis. *Dev. Biol.* **231**, 265–278.
- Harshman LG (1999) Investigation of the endocrine system in extended longevity lines of *Drosophila melanogaster*. *Exp. Gerontol.* **34**, 997–1006.
- Herman WS, Tatar M (2001) Juvenile hormone regulation of longevity in the migratory monarch butterfly. *Proc. Royal Soc., London* **268**, 2509–2514.
- Jones G, Jones D, Chu Y, Wozniak M, Xu Y, Fang F (2002) Ultrapiracle: A nuclear receptor that binds JH and JH-like structures and through which JH and JH-like structures can activate transcription. *45rd Annual Drosophila Research Conference, Abstract of Ecdysone Workshop*. Bethesda, MD: Genetics Society of America.
- Jones G, Sharp PA (1997) Ultraspiracle: An invertebrate nuclear receptor for juvenile hormones. *Proc. Natl Acad. Sci. USA* **94**, 13499–13503.
- Kelly TJ (1994) Endocrinology of vitellogenesis in *Drosophila melanogaster*. *Perspectives in Comparative Endocrinology*. National Research Council of Canada, pp. 282–290.
- Kozlova T, Thummel CS (2000) Steroid regulation of postembryonic development and reproduction in *Drosophila*. *Trends Endocrinol. Metabolism* **11**, 276–280.
- Pener MP (1972) The corpus allatum in adult acridids: the inter-relation of its functions and possible correlations with the life cycle. In *Proceedings of the International Study Conference on the Current and Future Problems of Acridology* (eds Hemming, CF, Taylor THC), pp. 135–147. London: Centre for Overseas Pest Research.
- Rankin SM, Stay B (1985) Ovarian inhibition of juvenile hormone synthesis in the viviparous cockroach, *Diploptera punctata*. *Gen. Compar. Endocrinol.* **59**, 230–237.
- Riehle MA, Brown MR (1999) Insulin stimulates ecdysteroid production through a conserved signaling cascade in the mosquito *Aedes aegypti*. *Insect Biochem. Mol. Biol.* **29**, 855–860.
- Salmon AB, Mark DB, Harshman LG (2001) A cost of reproduction in *Drosophila melanogaster*: stress susceptibility. *Evolution* **55**, 1600–1608.
- Soller M, Bownes M, Kubli E (1999) Control of oocyte maturation in sexually mature *Drosophila* females. *Dev. Biol.* **208**, 337–351.
- Tatar M, Kopelman A, Epstein D, Tu M-P, Yin C-M, Garofalo RS (2001b) A mutant *Drosophila* insulin receptor homolog that extends life-span and impairs neuroendocrine function. *Science* **292**, 107–110.
- Tatar M, Priest N, Chien S (2001a) Negligible senescence during reproductive diapause in *Drosophila melanogaster*. *Am. Natur.* **158**, 248–258.
- Tatar M, Yin C-M (2001) Slow aging during insect reproductive diapause: why butterflies, grasshoppers and flies are like worms. *Exp. Gerontol.* **336**, 723–738.
- Truman JW, Riddiford LM (2002) Endocrine insights into the evolution of metamorphosis in insects. *Annu. Rev. Entomol.* **47**, 467–500.
- Tu M-P, Epstein D, Tatar M (2002) The demography of slow aging in male and female *Drosophila* mutant for the insulin-receptor substrate homolog *chico*. *Aging Cell* **1**, 75–80.
- Warren TG, Mahowald AP (1979) Isolation and partial chemical characterization of the three major yolk polypeptides from *Drosophila melanogaster*. *Dev. Biol.* **68**, 130–139.