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Gonadal ecdysone titers are modulated by protein availability but do not impact protein appetite

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ABSTRACT

How animals survey internal nutrient availability to modulate specific appetites is currently largely unknown. Dietary proteins have a profound impact on the reproductive capacity and the selection of food sources in insects. When deprived of dietary proteins, insects stop producing eggs and develop strong protein appetites. In many adult insects, the ovaries are the site of synthesis of the ecdysone hormone. Therefore, an attractive hypothesis is that protein availability changes the gonadal production of ecdysone, which instructs the brain to increase its preference for yeast. We combine quantitative feeding assays, dietary manipulations, hormonal measurements, and genetic germline manipulations to test this hypothesis in *Drosophila melanogaster*. Our results show that upon yeast deprivation mated adult female *Drosophila* develop a strong yeast appetite and strongly reduce their egg production. This dietary manipulation also leads to a drastic reduction in ecdysone titers. However, the drop in ecdysone is not linked to the increase in yeast appetite as mutants with impaired oogenesis are able to adapt yeast intake to their nutrient state while displaying a constitutive low ecdysone titer. Interestingly, a low ecdysone titer is correlated with a lower level of overall food intake. Our data therefore show that in mated females the level of ecdysone reflects the level of protein in the diet and the physiological state of the ovaries. While the ovaries and ecdysone are unlikely to instruct the brain to develop a yeast appetite upon protein deprivation, they seem to be able to control overall food intake.

1. Introduction

Animals are constantly challenged to make decisions throughout their lifespan. In particular, feeding decisions are of paramount importance for the organism as the adequate supply of energy and other nutrients ensures its survival and reproduction. Homeostasis is a property of complex organisms allowing them to adapt to environmental fluctuations in the availability of resources (Leopold and Perrimon, 2007; Rajan and Perrimon, 2011). Accordingly, the coordination of nutrient intake and utilization is key to homeostasis and animals have evolved diverse behavioral repertoires to maintain adequate levels of nutrients (Corrales-Carvajal et al., 2016; Simpson and Raubenheimer, 2012).

Reproduction is highly dependent on nutrition, in particular, on amino acid (AA) availability (Drummond-Barbosa and Spradling, 2001; Hansen et al., 2004; Hosios et al., 2016; Leitão-Gonçalves et al., 2017; Piper et al., 2017). In *Drosophila melanogaster* for example, the availability of yeast, its main source of protein and AAs, is a key determinant of egg production (Bownes and Blair, 1986; Drummond-Barbosa and Spradling, 2001; Grandison et al., 2009). The fly adapts the rates of egg production to changes in yeast availability drastically and rapidly, within days (Drummond-Barbosa and Spradling, 2001). Ovaries from protein-deprived flies are greatly reduced in size, and their ovarioles contain few or no vitellogenic stage egg chambers (Drummond-Barbosa and Spradling, 2001; Schwartz et al., 1985; Terashima et al., 2005). Stem cells are especially sensitive to the diet. They respond to nutrient availability by modulating their proliferation rates and differentiation (Drummond-Barbosa and Spradling, 2001; Hsu and Drummond-Barbosa, 2009).

Animals have developed two different strategies to adapt food intake to their current internal state. One type of mechanism drives changes in food intake in a feed-forward, anticipatory way, increasing the intake of specific nutrients before the animal is completely deprived of them (Walker et al., 2017). One example of such a mechanism is the increase in salt and yeast intake mediated by the Sex Peptide upon mating in *Drosophila*. During copulation the male-derived Sex Peptide is transferred to the female and binds the neuronal expressed Sex Peptide Receptor SPR leading to an increase in yeast and salt feeding (Ribeiro and Dickson, 2010; Walker et al., 2015). At the circuit level SPR activation leads to the silencing of a dedicated neuronal circuit which

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projects to the central brain to change taste perception and hence the intake of specific nutrients (Corrales-Carvajal et al., 2016; Walker et al., 2015). Importantly, mating does not alter food choice by changing nutrient demand, since yeast and salt appetites are unaltered by the reproductive capacity of the animal (Ribeiro and Dickson, 2010; Walker et al., 2015). Another type of mechanism leads to changes in food intake via feed-back allowing the animal to adapt its behavior to compensate for specific nutrient deficits. As such, flies readily increase yeast intake upon yeast and AA deprivation (Corrales-Carvajal et al., 2016; Ribeiro and Dickson, 2010; Leitão-Gonçalves et al., 2017; Piper et al., 2014, 2017). The neuronal circuits and molecular pathways by which flies homeostatically increase yeast intake to compensate for a lack of AAs are not well understood. One possibility is that similarly to leptin in vertebrates, deficits in specific nutrients are measured in metabolically active tissues which lead to the secretion of a signaling molecule which acts on the brain to alter food intake (Myers et al., 2008). Alternatively, nutrients could be directly sensed by the nervous system to induce adaptive changes in food choice.

In adult female insects, the ovaries are the site of synthesis of multiple signaling molecules, including ecdysone. The hormonally active form of ecdysone, 20-hydroxyecdysone (20E), is produced from a cholesterol precursor derived from dietary yeast ergosterol. In *Drosophila* larvae ecdysteroids are produced in response to prothoracicotropic hormone (PTTH) and nutritional inputs (Colombani et al., 2005; Koyama et al., 2014; Mirth and Shingleton, 2014). The first steps of 20E synthesis occur in the larval prothoracic gland. Pulses of 20E are crucial for the timely development of the animal from the embryonic stage through the three larval instar stages into the adult fly. In the adult the titers of 20E are much lower when compared to earlier developmental stages (reviewed in Schwedes and Carney (2012)). In the adult female, 20E synthesis occurs in the ovaries (Bownes and Blair, 1986; Bownes et al., 1984; Domanitskaya et al., 2014; Hentze et al., 2013; Schwartz et al., 1985). Ecdysone has also been detected in other fly tissues, but it is currently unclear if it gets synthesized in these tissues (reviewed in Galikova et al. (2011), Schwedes and Carney (2012)). In the target tissues, 20E binds to a heterodimeric receptor consisting of EcR and Ultraspiracle, which activates a signaling cascade that alters the expression of target genes (reviewed in Galikova et al. (2011), Schwedes and Carney (2012)). While ecdysone is best known for its role in larval growth (reviewed in Schwedes and Carney (2012)), it is also thought to affect adult physiology regulating, among other things, vitellogenesis and egg production, courtship behavior, adult reproductive diapauses, innate immunity, as well as stress resistance and lifespan (reviewed in Galikova et al. (2011), Schwedes and Carney (2012)). Its function in controlling adult behavior however, still remains poorly explored.

Adult ecdysone levels appear to be responsive to changes in the environment. Titers of ecdysone are elevated in mated females, in males whose courtship advances have been rejected by females and in flies which have been sleep-deprived (reviewed in Schwedes and Carney (2012)). However, while there are reports showing that the availability of dietary yeast alters 20E titers, there is contradictory evidence on how it responds to this stimulus, with some reports suggesting an increase while others, a decrease upon yeast deprivation (Bownes, 1989; Schwartz et al., 1985; Terashima et al., 2005). Intriguingly, ecdysone signaling has been proposed to play an important role in adapting nutrient intake to the reproductive lipid demands of the female (Sieber and Spradling, 2015). Ovarian 20E levels could therefore play an important role in reporting the protein state of the female to the brain to adapt food choice in the adult fly.

In this study we aim at testing: a) if yeast deprivation leads to a change in ovarian ecdysone titers; and b) if ecdysone titers can act on the nervous system of the fly to direct changes in yeast appetite. To do so, we first tested flies that were either kept on a yeast-based medium (YBM) or a diet devoid of yeast for their feeding preference, ovary physiology and ecdysteroid titers. Protein deprived females show a drastic reduction in ovary size and a concomitant increase in yeast consumption compared to fully fed females. Furthermore, we observed a drastic reduction in ecdysone titers in yeast deprived females. To test if there is a causal link between the ecdysone decrease and the alteration of the fly's feeding preference, we used ovo^{D1} mutants which are severely impaired in egg production. Despite the ovaries of these mutant females being very small, these animals also increase yeast consumption after protein deprivation. However, ecdysone titers of these flies are drastically reduced, independently of the nutrient state of the female. Our data are therefore in agreement with ovaries being the prime site of ecdysone production in adults and their physiological state being a major determinant of adult female ecdysone levels. Furthermore, our data indicate that ecdysone is not likely to be involved in controlling food choice upon yeast deprivation, since ovo^{D1} females have constitutively low levels of this hormone without displaying any effect at the level of feeding decisions.

2. Materials and methods

2.1. Drosophila stocks and genetics

For all experiments w^{1118} mated females were used as the control genotype. ovo^{D1} mutant flies used in the experiments were generated by crossing males from the stock ovo^{D1} v^{24}/C(1)DX, y^1 w^1 f^1 (Bloomington #1309) (Oliver et al., 1987) to w^{1118} virgins. The resulting ovo^{D1} v^{24}/ w^{1118} females were termed ovo^{D1} mutants throughout the study.

2.2. Drosophila rearing, media, and dietary treatments

Flies were reared on yeast-based medium (YBM) (per liter of water: 8 g agar (NZYTech, PT), 80 g barley malt syrup (Prórida, PT), 22 g sugar beet syrup (Grafschafter, DE), 80 g corn flour (Prórida, PT), 10 g soya flour (A. Centazi, PT), 18 g instant yeast (Saf-instant, Lesaffre), 8 ml propionic acid (Argos), and 12 ml nipagin (Tegospet, Dutscher, UK) (15% in 96% ethanol) supplemented with instant yeast granules on surface (Saf-instant, Lesaffre). To ensure a homogenous density of offspring among experiments, fly cultures were always set with 6 females and 3 males per vial and left to lay eggs for 3 days. 14 days after the culture was started, flies were sorted and transferred to fresh YBM for 2 days and then transferred to fresh YBM for additional 24 h to ensure a well fed state. Subsequently, flies were either kept on YBM for 2 days with a final transfer to fresh food for 24 h (fully fed flies) or transferred to tubes containing paper towels soaked with 5 ml of 100 mM sucrose solution to induce protein deprivation for 3 days. Fly rearing, maintenance, and behavioral testing were performed at 25 °C in climate-controlled chambers at 70% relative humidity in a 12-hr-light-dark cycle (Aralab, FitoClima 60000EH).

2.3. flyPAD feeding assays

flyPAD assays were performed as described in Itskov et al. (2014). For food choice experiments, single flies in different dietary conditions were tested in arenas that contained two kinds of food patches: 10% Yeast and 20 mM Sucrose, each mixed with 1% agarose. Flies were individually transferred to flyPAD arenas by mouth aspiration and allowed to feed for 1 h at 25 °C, 70% relative humidity. The total number of sips per animal over this hour was calculated using previously described flyPAD algorithms (Itskov et al., 2014). Non-eating flies (defined as having fewer than 2 activity bouts during the assay) were excluded from the analysis.

2.4. 20E measurements

Ecdysteroid titers were measured using a protocol adapted from (Mirth et al., 2005; Porcheron et al., 1989a). 50 flies per condition were snap frozen in dry ice and stored at −80 °C awaiting further processing.