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The chemosensitivity of labellar sugar receptor in female *Phormia regina* is paralleled with ovary maturation: Effects of serotonin

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ABSTRACT

Oogenesis in most adult insects is a nutrient-dependent process involving ingestion of both proteins and carbohydrates that ultimately depends on peripheral input from chemoreceptors.

The main goal of this study was to characterize, in the female blowfly *Phormia regina*, the responsive changes of the labellar chemoreceptors to carbohydrates and proteins in relation to four different stages along the ovarian cycle: (1) immature ovaries, (2) mid-mature ovaries, (3) mature ovaries and ready for egg-laying and (4) post egg-laying ovaries. Then, the possible effects exerted by exogenous serotonin on the chemoreceptor sensitivity profiles were investigated.

Our results show that ovary length, width and contraction rate progressively increase from stage 1 to 3, when all these parameters reach their maximum values, before declining in the next stage 4.

The sensitivity of the labellar "sugar" chemoreceptors to both sucrose and proteins varies during the ovarian maturation stages, reaching a minimum for sucrose in stage 3, while that to proteins begins. Exogenous 5-HT supply specifically increases the chemoreceptor sensitivity to sugar at the stages 3 and 4, while it does not affect that to proteins.

In conclusion, our results provide evidence that in female blowflies the cyclic variations in the sensitivity of the labellar chemosensilla to sugars and proteins are time-related to ovarian development and that during the stages 3 and 4 the responsiveness of the sugar cell to sucrose is under serotonergic control.

1. Introduction

Oogenesis in most adult insects is a nutrient-dependent process involving ingestion of both proteins and carbohydrates (Wheeler, 1996). In all insects, the biosynthesis and deposition of yolk proteins into eggs during ovarian maturation is a prerequisite for reproductive success. This requires an exogenous source of proteins, with the brain–gut axis playing an essential role in the regulation of feeding control circuits. In the Diptera, the events involved in this circuitry initially begin with input arising from the fly's olfactory system, which are influenced by signal inputs from the brain that may be modulated by various biogenic amines. Once the fly has located the food source, biogenic amines again play a role in selective nutrient ingestion (i.e., carbohydrates versus proteinaceous nutrients) (Stoffolano et al., 2007). Thus, final ingestion depends on peripheral input signals from the contact chemoreceptors (Dethier, 1976). Accordingly, Simpson et al. (1991) reported that in locust the feeding behavior "was paralleled by nutrient-specific changes in gustatory responsiveness", thus suggesting that chemoreceptor responsiveness is influenced by hemolymphatic components.

Because of the already voluminous literature on both the reproductive (Yin and Stoffolano, 1990) and sensory system at the peripheral receptors (Dethier, 1976), the queen blowfly, *Phormia regina*, provides a model system to investigate the impact that carbohydrates and proteins have on the reproductive pathway leading to egg maturation.

The chemosensory taste system of the blowfly is composed of cuticular hairs, called sensilla, each containing four primary chemoreceptive neurons, three of which have been named "salt", "sugar" and "water" cells depending on their best stimuli. In fact, each neuron may also be responsive to other chemicals, such as amino acids (see for a review Dethier, 1976; Liscia et al., 1998).

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As for the remaining chemosensory cell, it has been named ‘‘deterrent’’ cell according to its responsiveness to deterrent compounds, similarly to the ‘‘bitter’’ cell in vertebrates (Liscia and Solari, 2000).

Many of the nervous pathways involved in regulating feeding have been characterized in the blowfly *P. regina*; in particular, it has been determined that regulatory pathways controlling protein and carbohydrate meal size may not overlap entirely (Dethier, 1961, 1976; Bernays and Simpson, 1982; Downer et al., 2007). In fact, insects satiated with a sucrose solution, and therefore not sensitive to high concentrations of sugar, may ingest protein despite being full. Thus, protein intake in non-blood feeders occurs also when the crop is full of sucrose and the abdomen is distended, showing that inhibition from stretch receptors is modified at the peripheral level to allow further food intake (Dethier and Gelperin, 1967; Gelperin, 1972; Bernays and Simpson, 1982). This non-overlap of the regulatory pathways controlling proteinaceous (e.g., here blood) and sugar intake also applies to blood-feeding insects (Stoffolano, 1983).

The relative ratio between carbohydrate and protein ingestion is not absolute, as suggested by Dethier (1961), and also depends on the stimulating effects exerted by these substances on single taste receptor cells. Therefore, the response of the taste chemosensory system influences the amount of food intake, but the receptor sensitivity also varies according to age, sex and hormonal state of the insects (Dethier, 1961; Stoffolano, 1973; Stoffolano et al., 1978; Angioy et al., 1983a,b; Blaney et al., 1986).

A protein meal triggers the neuroendocrine cascade leading to mating and to oogenesis. During the first stage of egg growth, the insect ingests large quantities of proteins, while after that their intake decreases (Stoffolano, 1974; Yin and Stoffolano, 1990, 1997). On the other hand, intake of carbohydrates, also used for maintenance, locomotion and flight (Dethier, 1976), is initially low and then increases (Strangways-Dixon, 1961). In the blowfly, it is known that the spike firing frequency of the labellar chemosensilla increases at the beginning of vitellogenesis, during which the release of juvenile hormone (JH) from the corpus allatum occurs (Pietra et al., 1978; Stoffolano, 1974; Stoffolano et al., 1978; Angioy et al., 1982, 1983a).

The previously held idea that feeding in *P. regina* was based mainly on sensory input, which was modulated by mechanical feedback from stretch receptors and receptor adaptation (Dethier, 1976) was changed by the research of Long and Murdock who clearly established the importance of the biogenic amines on carbohydrate feeding (Long and Murdock, 1983; Long et al., 1986; Brookhart et al., 1987). More information on the role of the biogenic amines on taste chemoreceptor activity, as it relates to egg maturation, needs to be considered. It is known that biogenic amines may act as neurotransmitters and neurohormones in the central nervous system (CNS) of insects. They are also released into the hemolymph where they act as neurohormones (Monastirioti, 1999).

Serotonin is a biogenic amine that can act as neurotransmitter and neurohormone both in vertebrate and invertebrate species: dipteran nervous and alimentary systems are well supplied with serotonergic cells and processes, and many of these nervous elements are of neurohormonal nature (Nässel and Elekes, 1985; Nässel, 1988; Pietrantonio et al., 2001; Dacks et al., 2003; Haselton et al., 2006, 2009; Menzel and Müller, 1996; Mercer et al., 1996; Yuan et al., 2005; Orchard, 2006). Many studies demonstrated that serotonin, and also octopamine, are involved in the regulation of circadian rhythms and motor activities (Cymborowski, 2003), in neural development, in memory and learning (Huser et al., 2012), as well as in various physiological processes, including the stimulation of salivary secretion by serotonin in the blowfly *Calliphora vicina* (via two recently identified 5-HT receptors; Röser et al., 2012) and in the cockroach (for a review, see Walz et al., 2006).

Moreover, in several dipteran species (Long and Murdock, 1983; Brookhart et al., 1987; Novak and Rowley, 1994; Dacks et al., 2003), injections of exogenous serotonin or serotonin agonists were shown to influence feeding by modulating tarsal acceptance thresholds and meal size.

Recently, it was shown that in adult blowflies, serotonin finely modulates the emptying rate of the crop, by increasing the overall contraction activity of this storage organ for carbohydrates, and therefore boosting the food transfer to the midgut for digestion (Liscia et al., 2012; Solari et al., 2013). On this basis, the goals of this study were to characterize, in the female blowfly *P. regina*, (1) the responsive changes of the labellar chemoreceptors to carbohydrates and proteins in relation to different stages along the ovarian cycle and (2) the possible effects exerted by exogenous serotonin on the chemoreceptor sensitivity profiles.

2. Materials and methods

2.1. Insects

Experiments were performed on adult female blowflies, *P. regina*, 1–6 days post-emergence, obtained from a colony maintained as previously described at 27 °C, 50% RH, and a photoperiod of 16L:8D (Stoffolano, 1974). Eggs were collected and placed in 473 ml plastic cups containing an artificial diet (Stoffolano et al., 2010). After several days of growth, the cups were placed into a container with sand; and, when the larvae were ready to pupate, they crawled into the sand. Pupae were then transferred to another 473 ml plastic cup, which was placed into a metal cage (20.3 cm on each side). When the adults emerged, they were fed a 0.126 M sucrose solution and had continual access to water and fresh beef liver according to Liscia et al. (2012). Liver is used both as a source of protein essential for mating and egg development and as an oviposition substrate. All flies emerging within 24 h were considered as one cohort.

2.2. Morphometry and contraction rate of ovaries at the different maturation stages

A study was made to evaluate ovarian growth and to identify four different stages of the ovarian cycle in females of *P. regina*. Female blowflies were cold-immobilized at −20 °C until they became inactive (typically less than 5 min). Using a dissecting stereomicroscope (Zeiss, Stemi 2000-C), they were restrained, ventral side up, in soft dental wax, according to the procedure used by Solari et al. (2013). A section of cuticle was removed from the ventral surface of the abdomen in order to expose the ovaries; dissections were performed in *Phormia* saline (composition in mM: NaCl 119, KCl 5.6, $CaCl_2{*}2H_2O$ 2.4, $MgCl_2{*}6H_2O$ 1, L-Glutamic acid 97, L-Glutamine 44, L-Proline 97, L-Alanine 48, Glycine 26, L-Histidine 6, pH 7.0, osmotic pressure 480 mOs/kg; Chen and Friedman, 1975) and care was taken to ensure that all abdominal organs, nerves and muscles remained intact.

The morphometry and the contraction frequency of the ovaries were video-recorded by way of a Moticam 2300 (3.0 M Pixel, USB 2.0; Motic, Hong Kong, China) color digital camera coupled to the stereomicroscope; video information was stored on a computer as avi files and analyzed with Motic Images Plus 2.0 ML software.

According to Giorgi et al. (1990) and Yin and Stoffolano (1997), on the basis of the yolk content of the follicles, ovaries were classified into the four following stages (Fig. 1): (1) immature ovaries (follicles did not contain yolk), (2) mid-mature ovaries (follicles were about $^1/_2$ full of yolk), but not completely full, (3) mature ovaries and follicles completely full of yolk and (4) ovaries after egg-laying. These 4 categories, based on follicle development, were