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Nutrition and dopamine: An intake of tyrosine in royal jelly can affect the brain levels of dopamine in male honeybees (*Apis mellifera* L.)

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ABSTRACT

Precursors of neuroactive substances can be obtained from dietary sources, which can affect the resulting production of such substances in the brain. In social species, an intake of the precursor in food could be controlled by social interactions. To test the effects of dietary tyrosine on the brain dopamine levels in social insect colonies, male and worker honeybees were fed tyrosine or royal jelly under experimental conditions and the brain levels of dopamine and its metabolite were then measured. The results showed that the levels of dopamine and its metabolite in the brains of 4- and 8-day-old workers and 8-day-old males were significantly higher in tyrosine-fed bees than in control bees, but the levels in 4-day-old males were not. The brain levels of dopamine and its metabolite in 4- and 8-day-old males and workers were significantly higher in royal jelly-fed bees than in control bees, except for one group of 4-day-old workers. Food exchanges with workers were observed in males during 1–3 days, but self-feedings were also during 5–7 days. These results suggest that the brain levels of dopamine in males can be controlled by an intake of tyrosine in food via exchanging food with nestmates and by self-feeding.

1. Introduction

The nutritional content of food can be an important factor affecting the production of hormones and neuroactive substances. Intake of a hormone precursor can supply a substrate for hormone synthesis and contribute to hormone production (Wurtman and Fernstrom, 1975; Fernstrom, 1977; Choi et al., 2009; Miller and Heyland, 2010). In generally solitary animals, food is acquired through foraging. By contrast, in social species, food is acquired through both foraging and food exchange with nest mates; thus, the nutritional status of their nestmates can be manipulated by those individuals that are involved in food exchange.

In animals, biogenic amines are neuroactive substances that transmit neural signals (neurotransmitters), modulate neuronal activities in local neural circuits (neuromodulators), and cause particular gene expressions in target cells (neurohormones) (Evans, 1980; Roeder, 1999; Lange, 2009). Biogenic amines, especially monoamines, are synthesized from specific amino acids, including tyrosine and tryptophan (Wurtman and Fernstrom, 1975; Evans, 1980; Livingstone and Temple 1983; Vaughan, 1988). Dopamine is a monoamine derived from tyrosine and has multiple roles in the control of movement, reinforcement, motivation, memory, arousal and reproduction in vertebrates and invertebrates (Wicker-Thomas and Hamann, 2008; Waddell, 2010; Perry and Barron, 2013; Nall and Sehgal, 2014), despite the differing structural organization of the central nervous systems (CNS) of these two groups. In social insects, including honeybees, dopamine is involved in the social system (Vergoz et al., 2007; Jurriault and Mercer, 2012) and regulates behavioral activities (Harano et al., 2008a; Akasaka et al., 2010) and female reproductive physiology (Boulay et al., 2001; Sasaki et al., 2009; Okada et al., 2015).

Although dopamine results from enzymatic processes in particular neurons in the CNS (Livingstone and Temple, 1983; Bicker, 1999), external or environmental factors can affect its production. However, those factors in social insects are still unclear. One known factor is the pheromone produced by the queen, which regulates the behavior of, and reproduction by, the workers (Jay, 1970; Vergoz et al., 2007; Jurriault and Mercer, 2012) and the brain levels of dopamine (Harris and Woodring, 1995; Sasaki and Nagao, 2001; Beggs et al., 2007). An additional external factor affecting dopamine levels is diet. Some foods contain tyrosine, which is a precursor of dopamine, as well as other amino acids that can be converted into tyrosine (Sasaki et al., 2012; Matsuyama et al., 2015). These external factors could depend on social interactions within, and nutritional condition of, the colony. Although their effects on the brain of worker honeybees and possibly of the queen are known (Sasaki et al., 2012), it is unclear whether they also have an effect on the brain of male honeybees.

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In male honeybees, juvenile hormone (JH) can work as an internal factor regulating the levels of dopamine in the brain (Harano et al., 2008b; Mezawa et al., 2013). JH in the hemolymph of males increases during sexual maturation (Tozetto et al., 1995; Giray and Robinson, 1996; Fahrbach et al., 1997) along with the levels of dopamine in the brain. Application of a JH analog can increase the level of dopamine in the brain of male honeybees, indicating that JH is in an upstream position in dopamine regulation. Such regulation has been reported in other hymenopterans, including males of the large carpenter bee (Sasaki and Nagao, 2013); it also expected to occur in female bumble bees (Röseler, 1977; Bloch et al., 2000) and paper wasps (Giray et al., 2005; Sasaki et al., 2007), based on the positive correlation between dopamine levels and JH titers during ovarian development. However, it remains to be determined whether brain dopamine in these species is singly regulated by JH in relation to age or internal cues, or by JH and external factors.

Dopamine promotes locomotor and flight activities during sexual maturation in honeybee males (Akasaka et al., 2010; Mezawa et al., 2013), which results in activation of the mating flight. Males might be able to change the levels of brain dopamine in response to social conditions, possibly enabling them to speed up or delay their behavioral maturation. This effect could also be manipulated by workers for the production of high-quality males in the colony under variable colony conditions. Tyrosine is contained in royal jelly (Townsend and Lucas, 1940; Haydak, 1970; Liming et al., 2009) and is consumed by all colony members. However, the amount of tyrosine available to the colony can depend on the food source and social conditions, in that the presence or absence of a queen and her brood in the colony results in the consumption or excess, respectively of royal jelly-like food produced by nurse bees. In the present study, I tested the effects of nutritional factors on levels of brain dopamine in male honeybees and compared them with those of worker honeybees. This can link to understanding of the behavioral control in males by workers in the colonies.

2. Materials and methods

2.1. Dietary treatments of tyrosine or royal jelly

Newly emerged males and workers of the honeybee (*Apis mellifera* L.) from four source colonies (C1–4) that contained naturally mated queens were used for the experiments. To collect the emerged males and workers, each brood comb containing pupae was removed from each source colony and incubated at 32 °C for 24 h. After incubation, the males and workers were marked on the thorax with paint and used for the experiments detailed below.

Fifteen of each of the marked males (0 days old), marked workers (0 days old), and unmarked nurse workers (unknown age) collected from the same source colony were kept in a wooden box (11.7 × 11.7 × 6.2 cm) covered with a steel net lid and with a floor coated in beeswax. A feeder made using seven plastic cups (id.: 9 mm, height: 10 mm) in a plastic dish (id.: 35 mm, height: 9 cm) was placed in the wooden box at a corner. For the tyrosine-fed treatments, each group of bees was provided *ad libitum* with either 1.0 mg/ml tyrosine in 40% sucrose solution (L-Tyr-fed), 2.0 mg/ml tyrosine in 40% sucrose solution (H-Tyr-fed), or 40% sucrose solution (control) and pollen cakes in an incubator at 32 °C for 4 or 8 days. For the royal jelly-fed treatments, each group was provided with a 1:1 mixture of royal jelly and 40% sucrose solution (Roy-fed) or 40% sucrose solution (control) and pollen cakes for 4 or 8 days. Royal jelly was collected via a commercial procedure using plastic cells from queenless colonies and stored at 4 °C until use in the experiment. The food was supplied in the feeder every morning. After 4 or 8 days of feeding, both male and worker honeybees were euthanized using liquid nitrogen and stored therein until HPLC-ECD analysis.

2.2. Measurements of dopamine and its metabolite in the honeybee brain

Frozen brains were dissected in ice-cold honeybee saline (128.33 mM NaCl, 2.68 mM KCl, 1.80 mM $CaCl_2$, pH 6.7) on a Peltier cooling unit (Kenis Ltd., Osaka, Japan) at approximately 4 °C under a microscope. Dissected brains were homogenized with a microglass homogenizer in 50 µl ice-cold 0.1 M perchloric acid containing 0.1 ng/µl 3,4-dihydroxyphenylacetic acid (DHBA) for 2 min. Each sample was then transferred into a 1.5-ml microcentrifuge tube and centrifuged at 20,600 g for 30 min at 4 °C. Supernatants were transferred to microvials for analysis by HPLC-ECD.

A HPLC-ECD system developed by Sasaki et al. (2012) was applied and used for the analyses of dopamine and *N*-acetyldopamine (NADA, a dopamine metabolite). The HPLC system comprised a solvent delivery pump, a refrigerated automatic injector, and a C18 reversed-phase column (250 × 4.6 mm id., 5 µm average particle size) maintained at 35 °C. An electrochemical detector set at 0.7 V was used under 35 °C. The mobile phase contained 0.18 M monochloroacetic acid and 40 µM 2Na-EDTA, which was adjusted to pH 3.6 with NaOH. Into this solution, 1.62 mM sodium-1-octanesulfonate and 5% CH_3CN were added. The flow rate was kept constant at 0.7 ml/min. External standards were run before and after the sample runs for the identification and quantification of dopamine and NADA. Each biogenic amine peak was identified by comparing both the retention time and hydrodynamic voltamograms with those of the standards. Measurements based on the peak height of the chromatograms were obtained by calculating the ratio of the peak height of a substance to the peak height of the standard.

2.3. Behavioral analyses of feeding and trophallactic interactions

Feeding behaviors on either royal jelly or sucrose, and any trophallactic interactions, including food begging and exchange, were recorded by a video camera in an incubator at 32 °C. The videos were recorded for 15 min after food supplementation on days 1, 2, and 3 days for the 4-day groups and on days 5, 6, and 7 days for the 8-day groups. The same individuals were used for the behavioral analyses and measurement of biogenic amines. Feeding behavior was defined based on four criteria: (i) extending the proboscis into the liquid food in the feeder; (ii) frequently touching the antennae to the food; (iii) twitching the abdomen during proboscis extension; and (iv) any of the above that lasted for at least 3 s. A trophallactic interaction was defined as: (i) touching of the proboscises of both bees; (ii) frequent contact of the antennae of both bees; and (iii) either of the previous behaviors lasting for at least 3 s. The frequency and duration of these behaviors were measured based on the video images. Individuals in the videos were sorted as males, marked workers, or unmarked workers.

2.4. Statistical analyses

In the tyrosine-fed experiments, data of the levels of brain dopamine and NADA in each age group within a colony were compared among treatments using a one-way analysis of variance (ANOVA) following the post-hoc test (Tukey-Kramer test, $P = 0.05$ significant level) for multiple comparisons. For the total data from two colonies, a three-way ANOVA was used to determine the factors (age, colony, and food) affecting the levels of brain dopamine and NADA.