General and Comparative Endocrinology xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen

Consumption of tyrosine in royal jelly increases brain levels of dopamine and tyramine and promotes transition from normal to reproductive workers in queenless honey bee colonies

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ARTICLE INFO

Article history:
Received 1 May 2014
Revised 22 October 2014
Accepted 4 November 2014
Available online xxxx

Keywords:
Biogenic amines
Division of labor
Nutrition
Polyphenism
Reproduction

ABSTRACT

Dopamine (DA) and tyramine (TA) have neurohormonal roles in the production of reproductive workers in queenless colonies of honey bees, but the regulation of these biogenic amines in the brain are still largely unclear. Nutrition is an important factor in promoting reproduction and might be involved in the regulation of these biogenic amines in the brain. To test this hypothesis, we examined the effect of oral treatments of tyrosine (Tyr; a common precursor of DA, TA and octopamine, and a component of royal jelly) in queenless workers and quantified the resulting production of biogenic amines. Tyrosine treatments enhanced the levels of DA, TA and their metabolites in the brain. Workers fed royal jelly had significantly larger brain levels of Tyr, DA, TA and the metabolites in the brains compared with those bees fed honey or sucrose (control). Treatment with Tyr also inhibited the behavior of workers outside of the hive and promoted ovarian development. These results suggest that there is a link between nutrition and the regulation of DA and TA in the brain to promote the production of reproductive workers in queenless honey bee colonies.

1. Introduction

Polyphenism is the discontinuous phenotypic plasticity of external morphology elicited by the environment, and is observed in many species across a range of animal taxa (Hartfelder and Emlen, 2005; Gilbert and Epel, 2009). The division of reproduction in social insects is a polyphenism caused by different nutritional conditions at the larval stage and results in morphological and behavioral specializations at the adult stage (de Wilde and Beetsma, 1982; O'Donnell, 1998; Evans and Wheeler, 2001; Hartfelder and Emlen, 2005; Page and Amdam, 2007). In highly eusocial species, including honey bees (*Apis mellifera* L.), reproduction is strongly biased toward queens, which have a high reproductive potential relative to workers, which are either sterile or have low reproductive potential (Winston, 1987). Such variation in behavioral and physiological states is a mechanism of adaptation to natural and/or social environments and is maintained by specific neurohormonal mechanisms.

Workers with low reproductive potential in many eusocial species can change their reproductive state and lay eggs under certain conditions, such as in the absence of a queen (Winston, 1987; Hartfelder and Emlen, 2005). This change in the reproductive state of workers is a tactic that enhances the reproductive success of the queenless colony and the individual queenless worker. The behavioral transition of normal workers to reproductive workers is partly mediated by neurohormonal activities in the brain. Brain biogenic amines, especially dopamine (DA) and tyramine (TA), are involved in this transition. Brain levels of DA, its metabolites [i.e. *N*-acetyldopamine (NADA) and norepinephrine (NE)] and TA are correlated with ovarian development (Harris and Woodring, 1995; Sasaki and Nagao, 2001, 2002), with brain levels of DA and DA-related substances being higher in queens than in workers (Brandes et al., 1990; Sasaki et al., 2012). Gene expression of several types of DA receptor and a TA receptor in the brain and ovarian tissue can influence the reproductive state of queenless workers (Beggs et al., 2007; Thompson et al., 2007; Vergoz et al., 2012), whereby DA and TA can accelerate ovarian development in reproductive workers (Dombroski et al., 2003; Sasaki and Harano, 2007; Salomon et al., 2012). Brain DA in queenless workers is regulated by several factors, including queen substances (Harris and Woodring, 1995; Sasaki and Nagao, 2001; Beggs et al., 2007) and

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http://dx.doi.org/10.1016/j.ygcen.2014.11.005
0016-6480/

TA (Sasaki and Harano, 2007). However, the regulatory systems that enhance the brain levels of DA and TA in queenless workers are still largely unknown.

Nutrition affects reproductive activities at adult stages in primitively and highly eusocial Hymenoptera (Markiewicz and O'Donnell, 2001; Hunt, 2007; Human et al., 2007). Reproductive individuals have higher expression of nutrient-sensing and growth-regulating genes, including insulin signaling-related genes (Toth et al., 2007, 2009; Okada et al., 2010). In queenless colonies of honey bees, workers with high nutritional states fed royal jelly or pollen can have developed ovaries (Lin et al., 1999; Human et al., 2007). Reproductive individuals in queenless colonies express the genes involved in the target of rapamycin and insulin-signaling pathways (Grozinger et al., 2007; Cardoen et al., 2011). Signaling of the epidermal growth factor receptor, which is activated by royalactin in royal jelly at the larval stage (Kamakura, 2011), mediates the transition of normal workers to reproductive individuals in queenless colonies (Formesyn et al., 2014). Thus, nutrition is a requirement for the transition of normal workers to reproductive individuals in queenless colonies in honey bees. Brain DA and TA could promote this transition (Dombroski et al., 2003; Sasaki and Harano, 2007; Salomon et al., 2012), but the relationship between the brain amines and nutrition has not been investigated. If DA and TA in the brain promote the reproduction of workers with low nutritional states in queenless colonies, the ovaries could not uptake sufficient yolk protein and not be developed. Therefore, the brain DA and TA may act on workers with high nutritional states, assuming that nutrition could be linked to the regulatory systems of DA and TA in the brain.

Tyrosine (Try), a common precursor of DA, TA and octopamine (OA) (see Fig. 1A), is contained in food of honey bees, including royal jelly as one of the 26 amino acids present (Townsend and Lucas, 1940; Haydak, 1970; Liming et al., 2009), although royal jelly contains at least 26 amino acids, of which Tyr is not the most abundant. Royal jelly is normally fed by nurse bees to the queen and larvae in the queenright colony, but in queenless colonies without broods, it can be shared among the workers. Therefore, reproductive individuals in queenless colonies might ingest a relatively large amount of Tyr by consuming royal jelly-like food. This intake of Tyr might enhance the levels of brain DA and TA in queenless workers and accelerate their transition from normal workers to reproductive individuals. In this study, we tested this hypothesis by determining the effect of oral application of Tyr or natural food, including royal jelly, on the levels of DA, TA and their metabolites in the brain, and the effects of Tyr on the reproductive state of workers in queenless colonies.

2. Materials and methods

2.1. Animals

Newly emerged honey bee workers (*Apis mellifera* L.) from six mother colonies that contained naturally mated queens were used for the experiments. To collect newly emerged workers, brood combs were removed from each mother colonies and incubated at 32 °C for 24 h. After incubation, 90–100 newly emerged workers were collected, marked on the thorax with paint and used for the experiments detailed below.

2.2. Cage experiment: dietary effects of Tyr or natural honey bee food on the brain levels of biogenic amines

Thirty emerged workers (0 days) were kept in a wooden box (11.7 cm × 11.7 cm × 6.2 cm) covered with a steel net lid and with a floor coated in beeswax. For Tyr treatment, each group of 30 workers was provided *ad libitum* with either 1.0 mg/ml Tyr in 40% sucrose solution, 2.0 mg/ml Tyr in 40% sucrose solution or 40% sucrose solution (control) and pollen cakes in an incubator at 30 °C for 8 days. For feeding of natural food (honey and royal jelly), each group of 30 workers was provided *ad libitum* with a 1:1 mixture of honey (Ho) and 40% sucrose solution, a 1:1 mixture of royal jelly (Roy) and 40% sucrose solution or 40% sucrose solution (control) and pollen cakes for 8 days. Honey was collected from uncapped honey cells in queenright colonies. Royal jelly was collected by a commercial procedure using plastic cells from queenless colonies and stored at 4 °C until use for the experiment. After 8 days of feeding, workers were euthanized using liquid nitrogen and stored therein until HPLC-ECD analysis. Workers from three different mother colonies (colony A–C) were used in the experiments.

2.3. Measurements of biogenic amines in the honey bee brain

Frozen brains were dissected in ice-cold honey bee saline (128.33 mM NaCl, 2.68 mM KCl, 1.80 mM $CaCl_2$, pH 6.7) on a dissecting dish under a microscope. Dissected brains were homogenized with a microglass homogenizer in 100 µl of ice-cold 0.1 M perchloric acid containing 100 ng/ml 3,4-dihydroxyphenylacetic acid (DHBA) for 2 min. Each sample was then transferred into a 1.5-ml microcentrifuge tube and centrifuged at 12,000 rpm for 30 min at 4 °C. Supernatants were transferred to microvials for analysis by HPLC-ECD.

Two HPLC-ECD systems were used for the analyses of biogenic amines and Tyr. For the analysis of biogenic amines, a HPLC-ECD system was applied that was developed by Nagao and Tanimura (1988, 1989). The HPLC system comprised a solvent delivery pump (EP-300, EICOM, Kyoto, Japan), a refrigerated automatic injector (231-401, Gilson, Middleton, WI, USA) and a C18 reversed-phase column (250 mm × 4.6 mm id., 5 µm average particle size, UG 120, Shiseido, Japan) maintained at 35 °C. An electrochemical detector set at 0.85 V was used under 35 °C. Signals from the electrochemical detector were recorded and integrated by using data analysis software (PowerChrom, ADInstrument, Castle Hill, NSW, Australia). 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 of sodium-1-octanesulfonate and 7% CH_3CN were added. The flow rate was kept constant at 0.7 ml/min. External standards were run before, midway through and after the sample runs for the identification and quantification of DA, NADA, TA, NATA and OA.

For the Tyr analysis, the HPLC-ECD system which is similar with the system for biogenic amines were used with a C18 reversed-phase column (MG 120, Shiseido), a detector maintained at 30 °C and data analysis software (EM Power, Waters, Milford, MA, USA). The mobile phase (pH 2.6) contained 83 mM citric acid monohydrate, 17 mM sodium acetate and 13 µM 2Na-EDTA. We added 2.3 mM sodium-1-octanesulfonate and methanol (7%) to this solution. The flow rate was kept constant at 0.7 ml/min. External standards were used for the identification and quantification of Tyr.

In both HPLC-ECD systems, each biogenic amine peak was identified by comparing both the retention time and hydrodynamic voltammograms with those of the standards. Measurements based on peak area of the chromatograms were obtained by calculating the ratio of the peak area of a substance to the peak area of the internal standard.

2.4. Colony experiment: effects of oral treatments of Tyr on workers staying in the hive and their ovarian development

For oral treatment with Tyr, marked workers were transferred into plastic cups (55 mm high × 75 mm id.) in group and provided

Please cite this article in press as: Matsuyama, S., et al. Consumption of tyrosine in royal jelly increases brain levels of dopamine and tyramine and promotes transition from normal to reproductive workers in queenless honey bee colonies. Gen. Comp. Endocrinol. (2014), http://dx.doi.org/10.1016/j.ygcen.2014.11.005