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Caste differences in dopamine-related substances and dopamine supply in the brains of honeybees (*Apis mellifera* L.)

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ABSTRACT

We determined the mechanisms underlying caste differences in the brain levels of dopamine-related substances in adult honeybees (*Apis mellifera* L.). Brain levels of dopamine, DOPA (a dopamine precursor), and *N*-acetyldopamine (a dopamine metabolite, NADA) were significantly higher in three-day-old virgin queens than same-aged workers. Caste differences in dopamine and NADA levels were also found in the hemolymph. The *in vitro* enzymatic activities of DOPA decarboxylase (DDC) during dopamine synthesis in brains were not significantly different between castes. The DDC activity in adult queens was mainly found in the brain, but with lower levels of activity detected in the mandibular glands, salivary glands and ovaries. Oral application of DOPA to workers led to DOPA uptake in the brain and significantly higher dopamine and NADA levels in the brain, suggesting that dopamine synthesis could be controlled by the amount of DDC substrate. Royal jelly samples taken from queen cells had a >25-fold higher concentration of dopamine compared with honey samples collected from honey cells. However, oral application of the same concentration of dopamine did not significantly enhance the brain levels of dopamine and NADA. These results suggest that the higher levels of brain dopamine in queens compared with workers can be explained by the higher level of DDC substrate, rather than DDC activity in the brain and other tissues of queens as well as exogenous dopamine in the royal jelly.

1. Introduction

Polyphenism is the discontinuous phenotypic plasticity of external morphology elicited by environments, which is observed in many species in a wide range of animal taxa [13,18]. The phase changes in locusts [29,44], seasonal changes in butterflies [9,39], and the division of labor in social insects [7,20] are also examples of polyphenism in insects. Variations in behavioral and physiological states that accompany polyphenism are adaptive to natural or social environments and they are maintained by specific neurohormonal mechanisms.

The division of reproduction in social insects is fundamental to the evolution and organization of insect societies. In highly eusocial societies, including honeybees (*Apis mellifera* L.), reproduction is strongly biased toward queens. These individuals have a high reproductive potential relative to workers that are either sterile or that have low reproductive potential. Caste-specific developmental pathways based on nutrition during the larval stages may, to some degree, determine the reproductive potential of an individual [22,45,46] and create a specialized adult brain that is morphologically and physiologically adapted to the performance of the behavioral tasks specific to its caste [3,14,24,41]. How the caste-specific behaviors of adults are physiologically regulated is an important issue in the division of labor in social insects. However, the neurohormonal mechanisms that interact with particular behavioral and physiological characteristics in the maintenance of social status are not well understood.

Biogenic amines are neuroactive substances that elicit behavioral and physiological differences in insects. They have roles as neurotransmitters, neuromodulators, and neurohormones in the peripheral and central nervous systems [12,23,31]. Biogenic amines also influence diverse physiological processes in insects, including the regulation of reproduction. In social Hymenoptera, biogenic amines, especially dopamine and tyramine, are involved in the reproductive maturation and reproductive behaviors of females (dopamine: [6,8,17,34]; tyramine: [32,33,35]). Brain dopamine may be involved in the behavioral and physiological transition of normal workers to reproductive workers in honeybees. It has been reported that brain levels of dopamine and its metabolite NADA are correlated with the reproductive status of workers [34]. Dopamine can accelerate the ovarian development of reproductive workers [11]. Both dopamine levels and dopamine receptor gene expression in the brains of queenless workers can be modulated by the queen mandibular pheromone [4,43]. Ovarian activations by dopamine have also been reported in the fire ant *Solenopsis*

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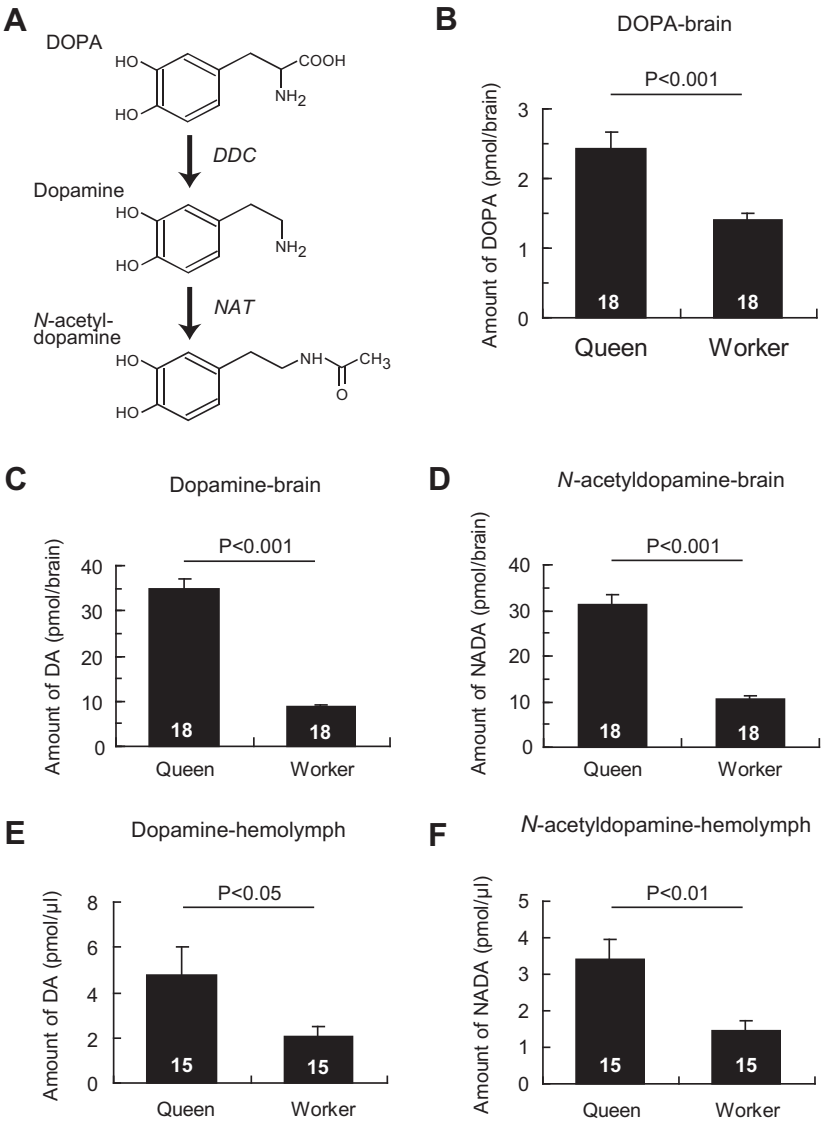

Fig. 1. Caste differences in dopamine-related substances in brains and hemolymph. (A) Dopamine synthetic and metabolic pathway in insects are shown. *DDC*: DOPA decarboxylase, *NAT*: *N*-acetyltransferase. Brain levels (mean ± SE) of DOPA (B), dopamine (C) and *N*-acetyldopamine (D) in queens and workers are indicated. Hemolymph levels of dopamine (E) and *N*-acetyldopamine (F) in queens and workers are also indicated. Three-day-old virgin queens and workers were used for the analysis. Sample sizes are indicated in each bar. Differences between castes were tested using the Mann–Whitney *U* test.

invicta [8] and the paper wasp *Polistes chinensis* [36,37]. These suggest that brain dopamine seems to be a key substance in the regulation of reproductive behavior in reproductive individuals of both primitive and highly eusocial Hymenoptera.

Caste differences in the brain levels of dopamine have been reported in eusocial bees [6,10]. In honeybees, the brain level of dopamine in the queen was 3.5–7 times higher than that in normal workers [10,15], although no large difference between castes was found in the number of dopamine secretory cells in the mushroom body [5]. Brain dopamine secretory cells are distributed widely throughout the mushroom body in this species [38,40], so the question arises of how the queen maintains a much higher concentration of brain dopamine compared with workers. Two major physiological mechanisms can be postulated as maintaining the caste differences in brain dopamine levels in honeybees. Firstly, there may be a difference between castes in terms of their production of endogenous dopamine from dopamine secretory cells. Greater enzymatic activity leading to dopamine synthesis in the dopamine secretory cells and/or lower enzymatic activity in dopamine conversion may be possible in queens, although the latter is unlikely because higher levels of dopamine metabolites have been reported in queens [10,15]. Dopamine synthesis via DOPA decarboxylase (DDC) activity (Fig. 1A) may occur in the brain and other tissues in queens, resulting in higher dopamine levels in the hemolymph and brains of these individuals. The substrate for DDC may control the brain dopamine levels, where larger amounts of a dopamine precursor (DOPA) may cause higher dopamine synthesis under constant DDC activities. However, no studies have compared the brain DDC activity in different castes or the activity of DDC in different tissues of queens. Even caste differences in hemolymph dopamine levels and brain DOPA levels have not been compared. The second mechanism by which caste differences in dopamine levels may be maintained is the additional supply of exogenous dopamine from the food, resulting in different levels in both the hemolymph and brain. Queens receive food known as "royal jelly" containing a high concentration of mandibular and hypopharyngeal gland secretions from nurse bees. The queens are provided with this food during the late larval periods and when