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Disturbances to neurotransmitter levels and their metabolic enzyme activity in a freshwater planarian exposed to cadmium

Jui-Pin Wu [a], Mei-Hui Li [a], Jhih-Sheng Chen [b], Szu-Yao Chung [b], Hui-Ling Lee [b,*]

[a] Environmental Toxicology Laboratory, Department of Geography, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei 106, Taiwan
[b] Department of Chemistry, Fu Jen Catholic University, 510, Zhongzheng Road, Xinzhuang District, New Taipei City 24205, Taiwan

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ABSTRACT

Using specific neurobehaviors as endpoints, previous studies suggested that planarian neurotransmission systems could be targets of Cd neurotoxicity. However, direct evidence for disturbed neurotransmission systems by Cd in treated planarians is still lacking. In planarians, dopamine (DA) and serotonin (5-HT) play critical roles in neuromuscular function, but little is known about their metabolic degradation. Therefore, in this study, we attempted to determine the appearances of DA, 5-HT, and their metabolic products in the freshwater planarian *Dugesia japonica*, characterize the activity of enzymes involved in their metabolism, and investigate the effects of Cd on planarian 5-HTergic and DAergic neurotransmission systems. Only DA, 5-HT, and 5-hydroxyindole-3-acetic acid (5-HIAA) were found in planarian tissues. Further enzymatic study revealed the activity of planarian monoamine oxidase (MAO) but not catechol-O-methyl transferase (COMT). These findings suggest that planarian MAO catalyzes the metabolism of 5-HT into 5-HIAA. However, DA metabolites from the MAO-involved metabolic pathway were not found, which might be due to a lack of COMT activity. Finally, in Cd-treated planarians, tissue levels of 5-HT and DA were decreased and MAO activity altered, suggesting that planarian neurotransmission systems are disturbed following Cd treatment.

1. Introduction

Although flatworms, or Platyhelminthes, are acoelomate and considered to be taxonomically and evolutionarily primitive, they are some of the simplest bilateral animals that possess a nervous system concentrated at the head end, which is considered the most primitive form of brain. Earlier studies revealed that some neurotransmitters found in higher animals also occurred in parasitic and free-living flatworms (Algeri et al., 1983; Buttarelli et al., 2008; Chou et al., 1972; Hariri, 1974; Lee et al., 1978; Ness et al., 1996; Ribeiro and Webb, 1984; Welsh and King, 1970). Thus, flatworms have been regarded as important animal models for neurology-related research, and in particular the freshwater planarians because they are relatively easy to maintain and manipulate experimentally in the laboratory.

Previously, we observed that cadmium (Cd) was found in the head of a treated freshwater planarian, *Dugesia japonica*, at a significantly higher concentration than the tail (Wu et al., 2011). Recently, concentration-dependent patterns in specific neurobehavioral responses of Cd-treated *D. japonica* were demonstrated (Wu et al., 2014). Accordingly, we suggested that the brain and nervous systems of planarians might be targets of Cd toxicity and that neurotransmission was possibly disturbed following exposure (Wu et al., 2014). However, a disturbance in planarian neurotransmission systems caused by Cd has still not been directly observed.

Dopamine (DA) and serotonin (5-HT) are two of the major neurotransmitters found in freshwater planarians. DA modulates the locomotor activity of planarians and 5-HT controls planarian regeneration and mediates the regulation of some neuromuscular functions (Carolei et al., 2008). Occurrences of both neurotransmitters and their synthetic pathways in planarians are known (Algeri et al., 1983; Ribeiro et al., 2005; Welsh and King, 1970).

Abbreviations: AAADC, aromatic amino acid decarboxylase; BA, benzylamine; CBN, nano-carbon black; Cd, cadmium; COMT, catechol-O-methyl transferase; CS, chitosan; DA, dopamine; DOPAC, 3,4-dihydroxyphenylacetic acid; FL, fluorescence; 5-HIAA, 5-hydroxyindole-3-acetic acid; HPLC–ECD, high performance liquid chromatography coupled with an electrochemical detector; HRP, horseradish peroxidase; 5-HT, serotonin; HVA, homovanillic acid; LC_{50}, median lethal concentration; L-DOPA, L-3,4-dihydroxyphenylalanine; MAO, monoamine oxidase; MAO-Is, MAO inhibitors; 3-MT, 3-methoxytyramine; PEA, phenethylamine; S-AMe, S-adenosyl-methionine; TA, tyramine; TH, tyrosine hydroxylase; TPH, tryptophan hydroxylase.

* Corresponding author. Tel.: +886 2 29053573; fax: +886 2 29023209.
E-mail addresses: rb5_wu@yahoo.com.tw (J.-P. Wu), meihuili@ntu.edu.tw (M.-H. Li), george7739@hotmail.com (J.-S. Chen), csy12190115@hotmail.com (S.-Y. Chung), 076308@mail.fju.edu.tw (H.-L. Lee).

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In contrast, neither of the metabolic pathways of DA and 5-HT have been investigated nor their metabolites identified in freshwater planarians. In most animals, DA metabolism is mainly catalyzed by monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT), while MAO also catalyzes the metabolism of 5-HT (Fig. 1). However, their activities have not also been characterized in freshwater planarians until now.

Therefore, the objectives of this study were (1) to determine the appearance of DA, 5-HT, and their metabolic products in the freshwater planarian *D. japonica*, (2) characterize MAO and COMT in *D. japonica* and measure their activity to reveal the metabolic pathways of 5-HT and DA in freshwater planarians, and (3) investigate the effects of Cd on 5-HTergic and DAergic neurotransmission systems in treated *D. japonica*. Planarians have one obvious neuronal cluster, or brain, located at the head end and two symmetric longitudinal cords with ladder-like interconnections running the length of the animal (Nishimura et al., 2008). Thus, in this study, we decapitated planarians into two fragments, head and tail, and used them to represent their central (brain) and peripheral nervous systems, respectively. The levels of 5-HT and DA and activity of their metabolic enzymes in these Cd-treated planarian fragments were measured.

2. Materials and methods

2.1. Chemicals

Cadmium sulfate ($3CdSO_4{\cdot}8H_2O$) was purchased from Mallinckrodt (Hazelwood, MO, USA). Nano-carbon black (CBN; particle size = 13 nm) was purchased from UniRegion Bio-Tech (Taiwan). Amplex® Red reagent was obtained from Life Technologies Corporation (Carlsbad, CA, USA). Other chemicals were purchased from Sigma–Aldrich (St Louis, MO, USA), including L-3,4-dihydroxyphenylalanine (L-DOPA), 3,4-dihydroxyphenylacetic acid (DOPAC), DA, 5-hydroxyindole-3-acetic acid (5-HIAA), homovanillic acid (HVA), 5-HT, chitosan (CS), tyramine (TA), benzylamine (BA), phenethylamine (PEA), clorgyline, pargyline, and others. All chemicals used were at A.C.S. or analytical grade.

2.2. Animals

The stock of *D. japonica* used in this study was obtained from Taikong Corporation (Taipei, Taiwan) and acclimated in our laboratory for more than two weeks as described previously (Wu et al., 2012). Size-selected intact planarians (~1 cm TL) were used for this study. Planarians were starved for one week to create a uniform metabolic status before starting the experiments.

2.3. Preparation of catecholamine standards

Stock solutions of different catecholamines at 10 mM concentrations were prepared by dissolving catecholamines in 0.1 N perchloric acid and stored at −20 °C. Before use, catecholamines were freshly diluted to desired concentrations with methanol–phosphate buffer solution (0.05 mol/L, pH 6.8) (5:95, v/v).

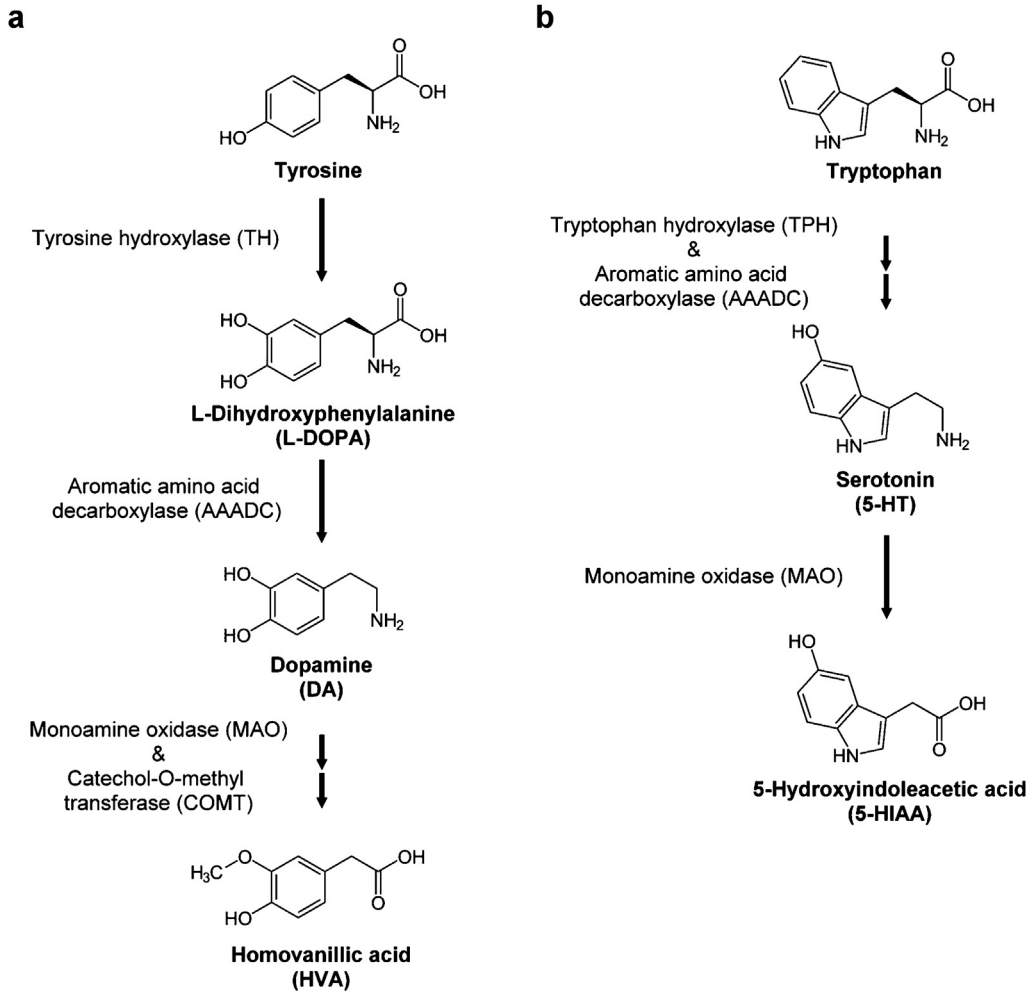

Fig. 1. Biosynthesis and metabolic pathways of (a) dopamine (DA) and (b) serotonin (5-HT) in vertebrates.