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Prevention of progeny formation in *Drosophila melanogaster* by 1-arylimidazole-2(3*H*)-thiones

Akinori Hirashima [a,*], Manabu Matsushita [b], Hiroto Ohta [b], Kentaro Nakazono [b], Eiichi Kuwano [a], Morifusa Eto [c]

[a] *Department of Applied Genetics and Pest Management, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*
[b] *Graduate School of Bioresource and Bioenvironmental Sciences, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan*
[c] *Professor Emeritus of Kyushu University, 7-32-2 Aoba, Higashi-ku, Fukuoka 813-0025, Japan*

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Abstract

Effects of 1-arylimidazole-2(3*H*)-thiones (AITs) and 1-(substituted benzyl)imidazole-2(3*H*)-thiones (BITs) were tested on progeny formation in *Drosophila melanogaster*. Some AITs showed inhibitory activities at laying eggs and delayed eclosion by 1 day. The inhibitory activity was nullified by adding octopamine (OA) or noradrenaline (NA) to the medium for progeny formation in *D. melanogaster.* The effect of AIT on the contents of OA and NA was analyzed in adults of *D. melanogaster* by high-performance liquid chromatography with electrochemical detection. Flies fed with AIT decreased OA and NA levels and increased TA content. Taken together, the inhibitory activity of AIT could be due to inhibition of tyramine β-hydroxylase and dopamine β-hydroxylase.
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1. Introduction

It has been demonstrated that biogenic amines control energy metabolism in insects and regulate the secretion of other hormones. Octopamine (OA) plays a key role as neurotransmitters, neurohormones, and neuromodulators in invertebrate systems [1] with a physiological role analogous to adrenaline in vertebrates [1–3]. Catecholamines were reported to play an important role in insects not only as neurotransmitters in nervous system and also as cross-linking agents in cuticle sclerotization and tanning [1,4]. Dopamine (DA) and noradrenaline (NA) seem to be the principal catecholamines in insects [1,5]. Hodgetts and Konopka [6] failed to detect any NA in flies. However, the reports of Tunnicliff et al. [7] and O'Dell et al. [8] do support the presence of NA in *Drosophila*. Watson et al. [9] have measured the content of NA in the heads of flies of *D. melanogaster* and have established that the content of the amine is very low in fruit fly. They showed that DA content is 11 times higher than the titer of NA in the flies of wild type line Canton-S of *D. melanogaster* [9]. The concentrations of DA and OA are similar in the cockroach *Periplaneta americana*, while NA is present in very low amount [10,11].

OA biosynthesis requires tyramine β-hydroxylase (TβH) to convert tyramine (TA) to OA. In *D. melanogaster*, the creation of null mutations at the TβH locus results in complete absence of the OA biosynthesis and TβH-null female flies are sterile, although they mate normally. This defect in egg laying is associated with the OA deficit, because females that have retained eggs initiate egg laying when transferred onto OA-supplemented food [12]. Studies in locusts have shown that OA has been

* Corresponding author. Fax: +81 92 642 2858.
E-mail address: ahirasim@agr.kyushu-u.ac.jp (A. Hirashima).

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implicated in the modulation of oviductal visceral muscle by two lines of evidence: innervation of the oviductal muscle by octopaminergic median unpaired neurons [13] and physiological evidence that OA modulates activity of the oviductal muscle [14]. The inhibition of dopamine-β-hydroxylase (DβH), the enzyme converting DA into NA, had significant effects on prevention of progeny formation in *Drosophila* [15], suggesting that reproduction in *Drosophila* is dependent upon NA. We present here, the effects of an inhibitor of DβH on progeny formation and the contents of NA, OA, and its precursor TA in *D. melanogaster*, using a simple method based on high-performance liquid chromatography (HPLC) with electrochemical detector (ECD). Furthermore, present report determines whether the prevention of progeny formation is due to inhibition of DβH or TβH.

2. Materials and methods

2.1. Chemicals

All 1-arylimidazole-2(3*H*)-thiones (AITs, **1**–**8**) and 1-(substituted benzyl)imidazole-2(3*H*)-thiones (BITs) were prepared by the condensation of the corresponding arylisothiocyanates and substituted benzylisothiocyanates with aminoacetaldehyde dimethyl acetal followed by acid-catalyzed cyclization of the intermediate *N*-arylthioureas and *N*-(substituted benzyl)thioureas [16,17]. AITs and BITs are reported to inhibit DβH [16].

2.2. Insects and fertility assay

Wild type *D. melanogaster* stock flies (Canton-S) were maintained by routine transfer on the standard nutrient medium at 25 °C and 60% RH at a density of 25 larvae per 10 ml of medium under a 18 h light:6 h dark photoperiod. The medium comprised 100 g of glucose, 50 g of dried yeast, 70 g of corn meal, 6 g of agar, 4 ml of propionic acid, and 10 ml of nipagin M (10 g of methyl *p*-hydroxybenzoate dissolved in 100 ml of 99% EtOH) per liter of distilled water. One virgin female and three males were placed in a vial (3 cm Ø × 10 cm) with the medium containing test compounds. They were allowed to lay eggs for 6 days, parents being transferred every day to new vials containing the medium. The progeny formation was determined by counting the total number of emerged flies for 19 days. Values are means of eight vials with SE. Flies were collected 24 h after emergence, frozen in liquid nitrogen, and stored at −85 °C to measure whole-body concentrations and contents of the brain biogenic amines.

2.3. HPLC

Brains of flies were dissected in phosphate buffered saline (0.01 M; NaCl 0.138 M; KCl 0.0027 M). Whole-body flies or brains were homogenized in 50 μl of ice-cold 0.1 M perchloric acid (PCA) containing 200 μM EDTA and 3,4-dihydroxybenzylamine (DHBA; 10 ng/ml) as an internal standard in an Eppendorf polypropylene microtube (1.5 ml) using a chilled microtube homogenizer (S-203, Ikeda Sci., Tokyo, Japan). The homogenate was centrifuged at 12,000*g* and 4 °C for 30 min. The supernatant was filtered through a filter (0.2 μm, HLC-DISK 13; Kanto Chemical, Tokyo, Japan) and 10 μl were injected directly for the measurement of OA and TA into an HPLC column through a valve (7125; Rheodyne, Cotati, CA) fitted with a 20-μl sample loop. For NA analysis, the filtrate was transferred to an Eppendorf polypropylene microtube (1.5 ml) along with 30 mg active Al_2O_3 in 1.5 times of Tris buffer (pH 8.3) against the filtrate and vortexed vigorously. After being stood for a couple of minutes, solution was discarded and the Al_2O_3 was washed with 500 μl of distilled water. The water was decanted and to the tube added ice-cold 0.1 M perchloric acid (PCA) containing 200 μM EDTA and 3,4-dihydroxybenzylamine (DHBA; 10 ng/ml) as an internal standard.

Chromatography was carried out in a reverse-phase column (4.6 × 250 mm) protected by a precolumn (4.6 × 10 mm) packed with Cosmosil 5 C_{18} AR of 5-μm average particle size (Nacalai Tesque, Kyoto, Japan) maintained at 25.0 ± 0.1 °C in a column oven. Separated compounds were detected simultaneously by an ECD-300 (Eicom, Kyoto, Japan) with an amperometric graphite electrode set at an oxidizing potential of 400 mV for NA and 850 mV for OA and TA (relative to an Ag/AgCl reference electrode). It has been shown that the detection sensitivity increases with the potential applied to the electrode, until the response reaches the plateau region. However, a higher applied potential results in an increase in the background current. In order to maintain good selectivity, sensitivity, and stability of detection, the detector should be worked at the lowest oxidation potential. Based on the results of hydrodynamic voltammograms, the detector potential for oxidative analysis was maintained at 400 mV for NA and 850 mV for OA and TA versus an Ag/AgCl reference electrode. Signals from the ECD were recorded and integrated by a data processor, PowerChrom (v.2.2.4; Bio-Research Center, Nagoya, Japan). Mobile phase was filtered under vacuum through a 0.45 μm pore filter (Millipore SJLHM4710) to remove the particulate matter. The flow rate was maintained at 0.8 ml/min. Concentration of biogenic amines were calculated by comparing the ratios, based on the peak height of chromatogram between the samples and standard DHBA.

2.4. Statistical analysis

In the experiments, differences between two treatments were compared by Student's *t* test and that among three or more treatments were analysed by One-way Factorial ANOVA followed by Scheffe test as post-hoc test. All differences, unless otherwise noted, are reported at $p < 0.05$.

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