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Evidence for a Biogenic Amine Pathway in the Plant-parasitic Nematode *Ditylenchus dipsaci*

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A number of studies have reported the presence of dopamine in free-living and animal-parasitic nematodes (6), but, to our knowledge, *Aphelenchus avenae* is the only plant-parasitic nematode in which this neurotransmitter has been shown by direct assay of tissue homogenates (7). This paper presents experimental evidence of the existence of biochemical pathways leading to the production of dopamine in *Ditylenchus dipsaci,* thus suggesting that dopamine is a ubiquitous neurotransmitter in a wide range of economically important plant-parasitic nematodes.

Preparation of nematode homogenates: Ditylenchus dipsaci was cultured on alfalfa callus tissues grown on nutrient agar at 21 C following Krusberg's procedure (3). The nematodes were harvested from 6–8-week-old cultures by a modified Baermann funnel technique under sterile conditions at 25 C for 2 days. The collected nematodes were then concentrated by centrifugation at 8,000 *g* for 5 minutes and washed twice with distilled water before homogenization.

Water-packed nematodes (ca. 2.5 ml) were suspended in 5 ml phosphate buffer pH 6.8 and homogenized in a glass tissue grinder with the aid of glass powder for 30 minutes in an ice bath. The homogenate was centrifuged at 15,000 *g* for 15 minutes at 0 C. The supernatant served as the crude nematode extract and was used immediately.

Incubation of reaction mixture: The procedures for radiolabelling the reaction mixture, thin layer chromatography, and the autoradiographic detection of labelled compounds were as described in a study of biogenic amines of *Caenorhabditis briggsae* by Kisiel et al. (2). As received, the labelled tyrosine contained small amounts of several radioactive impurities. The radiolabelled material was purified before use by the procedure described by Zuckerman et al. (8). The reaction mixture, consisting of 2.83 μCi L-[U-^{14}C] tyrosine (specific activity 405 mCi (millicurie)/mM), 0.05 M unlabelled tyrosine (10 μl), 0.1 M phosphate buffer pH 6.8 (1 ml), and 1 ml nematode extract, was incubated at 20 C. Nematode extract (1 ml) boiled for 15 minutes was the control. After incubation for 1 hour, the enzyme reaction was stopped by adding 2.5 ml boiling 95% ethanol, and the mixture was centrifuged at 15,000 *g* for 10 minutes to remove precipitated protein. The supernatant obtained was concentrated to 1 ml by freeze-drying. The [^{14}C]CO_2 evolved during the reaction was collected and bubbled through $Ba(OH)_2$, and the resultant [^{14}C] $BaCO_3$ was assayed in a Q-gas counter (counting efficiency 20%).

Thin-layer chromatography: Thin-layer chromatography was carried out on glass plates coated with a 250-μm film of Silica Gel G. In each case a 10-μl subsample of the reaction products was spotted both atop and adjacent to the unlabelled standard. Therefore, when the concentration of a reaction product was below that detectable by the spray reagent, its presence was confirmed by the coincidence of the labelled product and the unlabelled standard. In preliminary experiments 12 replicate plates were developed by one-dimensional chromatography utilizing butanol-acetic acid-water (4:1:5) as the solvent. The final trials consisted of four replicate plates developed by two-dimensional chromatography using butanol-acetic acid-water (4:1:5) for the first

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run and phenol-water (3:1) for the second. Autoradiograms were made using no-screen X-ray films. Plates were sprayed with either 0.3% ninhydrin in butanol (to detect amino acids) or diazotized sulfanilic acid (for phenolics). The retardation factors of labelled products obtained from metabolic reactions were checked against standards of the following compounds: L-tyrosine, L-phenylalanine, 3-monoiodo-L-tyrosine, 3,5-diiodo-L-tyrosine, L-3,4-dihydroxyphenylalanine (DOPA), dopamine, L-hydroxyphenylpyruvic acid, homovanillic acid, homogentisic acid, 3,4-dihydroxyphenylacetic acid, tyramine, 3,3',5-triiodo-L-thyronine, DL-3,4-dihydroxymandelic acid. Radioactive spots were eluted with 10% isopropanol and counted in a liquid scintillation counter (counting efficiency 80%).

Results and discussion: More than half of the L[U-^{14}C] tyrosine substrate was metabolized by the *D. dipsaci* extract. The three labelled reaction products identified were tyramine, dopamine, and DOPA. The relative amounts of each of these compounds were based on counts of radioactivity (Table 1). Small amounts of five other reaction products were detected, but these were not identified. The decarboxylation of tyrosine to tryamine was indicated by the evolution of [^{14}C]CO^2 recovered as [^{14}C] $BaCO_3$ (Table 1). The boiled extract contained only the original labelled substrate.

The three compounds formed from tyrosine are important precursors of two catechol pathways. One pathway leads to the formation of hormonal compounds (i.e., norepinephrine and epinephrine) and the other to pigments (melanin). Experiments with *C. briggsae* in which ^{14}C-labelled tyramine was used as the substrate demonstrated the presence of both epinephrine and norepinephrine (2). These hormones would not have been detected in this study because they would have been precipitated by the addition of boiling ethanol.

The results indicate that *D. dipsaci* can convert tyrosine to dopamine utilizing either of two biosynthetic pathways: tyrosine → tyramine → dopamine or tyrosine → DOPA → dopamine (5). The presence of tyrosine decarboxylase was suggested by the recovery of ^{14}C-labelled barium carbonate from a CO_2 trap.

TABLE 1. *Ditylenchus dipsaci*: Tyrosine and its metabolic products as determined by retardation factor (Rf) values in two solvents and radioactive counts.

Compound	Rf value†		Recovered radioactivity ($\times 10^{-4}$)‡
	Butanol-acetic acid-water	Phenol-water	
Tyrosine	0.35	0.48	62.5
Tyramine	0.51	0.66	41.7
DOPA	0.27	0.39	19.2
Dopamine	0.39	0.62	7.8
Unidentified			9.7
Ba[^{14}C]CO_3			2.8

† Average values from four two-dimensional TLC plates.
‡ DPM minus background.

Catecholaminergic neural structures were demonstrated in *Xiphinema americanum* (1) utilizing FIF-induced fluorescence, but this histochemical procedure does not allow for distinction between biogenic amines. A limiting factor in chemical definition of biogenic amines in plant-parasitic nematodes is obtaining a sufficient biomass for analysis. The required amount of biological material can be accumulated using monoxenic plant callus culture (3), but many of the important plant parasites cannot be grown by this method. Immunolabelling with antibodies against specific neurotransmitters appears to be the most promising approach to localizing neural sites of biogenic amines in plant-parasitic nematodes (4).

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