Dopamine and octopamine in whole body extracts of the bulb mite

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Summary. Using high performance liquid chromatography with electrochemical detection, whole body extracts of the bulb mite, *Rhizoglyphus echinopus* (Fumouze and Robin), were found to contain the biogenic amines dopamine and octopamine at concentrations of 4.3 ± 0.6 and 2.3 ± 1.4 ng g^{-1} wet weight, respectively. Adrenaline, noradrenaline, tyramine, *N*-methyldopamine, *N*-acetyldopamine, and 5-hydroxytryptamine, if present, were below the limits of detectability. This is the initial demonstration of the presence of octopamine in a mite species.
Key words. Biogenic amines; dopamine; octopamine: bulb mites.

Biogenic amine function in animals continues to be a subject of intense research. Such studies are facilitated by a knowledge of which biogenic amines are present in a specific organism. The presence of various amines has been demonstrated in many invertebrates[1–6]. However, in the Acari (ticks and mites) few biogenic amines have been identified. Noradrenaline and dopamine have been found in ticks[7,8], and only dopamine has been reported from mites[9,10]. Moreover, in this latter case, the concentration of dopamine in mites was likely overestimated. In this study we report the concentrations of dopamine and octopamine in the bulb mite, *Rhizoglyphus echinopus* (Fumouze and Robin).

Materials and methods

Bulb mites were reared at high humidity in darkness at 26–27 °C in petri dishes containing a wheat germ based diet[11]. The desired quantity of mites, usually 1–3 g, was homogenized on ice in a mixture of 4 ml of 0.4 N perchloric acid (PCA), 0.25 ml of a 1% solution of sodium metabisulfite, and 0.5 ml of a 1% solution of disodium ethylenediamine tetraacetic acid (EDTA). The homogenate was centrifuged at 20,000×g for 20 min at 4 °C, and pH of the supernatant was adjusted to 6.5 with potassium carbonate. The supernatant was subjected to ion exchange chromatography on Amberlite IRP-64 (hydrogen form, wet mesh 100–500) (Sigma Chemical Co., St. Louis, MO, USA) that had been previously washed and activated[12,13]. The column was rinsed sequentially with 1 ml of 0.01 N HCl containing sodium metabisulfite (0.5 g l^{-1}) and EDTA (0.5 g l^{-1}), 2 ml of 0.1 M sodium acetate (pH 4.2), and 0.5 ml of 2 M ammonium sulfate. Monoamines were eluted with 1.5 ml of 2 M ammonium sulfate. The ammonium sulfate fraction was adjusted to pH 8.6 with Tris buffer, and about 25 mg of activated acid-washed alumina (Sigma) were added. The sample was vortexed, and the alumina was sedimented by centrifugation. The supernatant, which contained the octopamine, was collected for subsequent analysis. The catecholamines were desorbed from the alumina with 0.4 N PCA. The sample was centrifuged at 20,000 g for 20 min at 4 °C, and the supernatant was analyzed for adrenaline, noradrenaline, dopamine, tyramine, *N*-methyldopamine, and *N*-acetyldopamine (all from Sigma) by HPLC as described below. The octopamine (Sigma)-containing supernatant from the alumina treatment was loaded onto a 3-ml Baker C_{18} disposable column (Rainin Instrument Co., Inc., Woburn, MA, USA) containing 500 mg of activated sorbent, and the column was rinsed with 1 ml of water followed by 0.5 ml of methanol containing 20 mM trichloroacetic acid (TCA)[14]. The methanol-TCA eluate was evaporated to dryness at 60 °C under nitrogen. The residue was dissolved in 0.1 ml of 0.4 N PCA and filtered through microfilterfuge tubes (Rainin) at 10,000 g for 5 min. The filtrate was analyzed by HPLC for octopamine as described below.

For HPLC a Waters pump system (Waters Associates, Milford, MA, USA) fitted with a 0.5 µ octadecyl column (4.5 × 150 mm) (III Supplies Co., Meriden, CT, USA) was connected to a single electrode amperometric electrochemical detector (Bioanalytical Systems, Inc., West Lafayette, IN, USA) set at 0.65 eV for catecholamines/5-hydroxytryptamine or to a dual electrode detector (Bioanalytical Systems) set at 0.65 and 0.50 eV for octopamine. The solvent system, which was modified after Scott et al.[15], had a pH of 6.0 and contained the following per liter of double distilled water: 8.2 g of sodium phosphate dibasic; 4.2 g of sodium citrate monohydrate; 40 mg of sodium octyl sulfate; and 20 mg of EDTA. For catecholamines/5-hydroxytryptamine analyses, the solvent system contained 2% methanol, whereas no methanol was used in octopamine analyses. For catecholamine/5-hydroxytryptamine analyses, the internal standard was 3,5-dihydroxybenzylamine (Sigma), whereas octopamine recoveries were determined by processing standard octopamine under identical conditions as mite samples. Total recoveries were between 40 and 50% with about 70% recovery in the ion exchange clean-up and about 70% recovery in the alumina extraction (catecholamines/5-hydroxytryptamine) or C_{18} clean-up (octopamine). Peak height was used for quantitation of biogenic amines, and concentrations reported were corrected for recoveries.

Results and discussion

Of the biogenic amines listed in the table, only dopamine and octopamine were present in concentrations exceeding the limits of detectability. Dopamine was positively identified by cochromatography with the authentic amine as shown in figure 1. Other experiments using different concentrations of the dopamine standard corroborated the presence of this amine in the mites. Interestingly dopamine also was present in the diet at about half of the concentration (wet weight) found in the mites

Biogenic amines isolated from bulb mites

Biogenic amine	Concentration ng g^{-1} wet wt[a]
Dopamine	4.3 (0.6)
Octopamine	2.3 (1.4)
N-Methyldopamine	< 2.1[c]
Tyramine	< 1.2[c]
Adrenaline	< 0.1[c]
Noradrenaline	< 0.4[c]
N-Acetyldopamine	< 0.7[c]
5-Hydroxytryptamine[b]	< 0.5[c]

[a] Mean and standard deviation, n = 3. [b] Chromatographed after ion exchange chromatography and prior to the alumina clean-up. [c] Lower level of detectability.

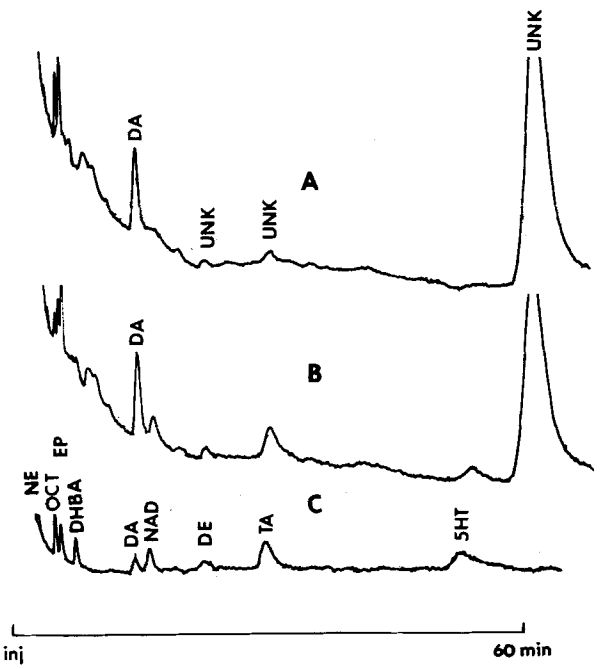

Figure 1. Chromatograms of bulb mites (3 g wet wt equivalent) whole body extract after ion exchange and alumina clean-up. *A* Mite extract only; *B* mite extract plus amine standards; and *C* amine standards only. Abbreviations: NE, noradrenaline; OCT, octopamine; EP, adrenaline; DHBA, dihydroxybenzylamine; DA, dopamine; NAD, *N*-acetyldopamine; DE, *N*-methyldopamine; TA, tyramine; 5HT, 5-hydroxytryptamine; and UNK, unknown. Although OCT and 5HT were included in the standards, their presence in this fraction of the mite extract was not expected since neither of these two amines would have been adsorbed by the alumina. Conditions: 50 µl injection; 1 ml min^{-1} flow rate; electrode 0.65 eV; and 5 nA full scale.

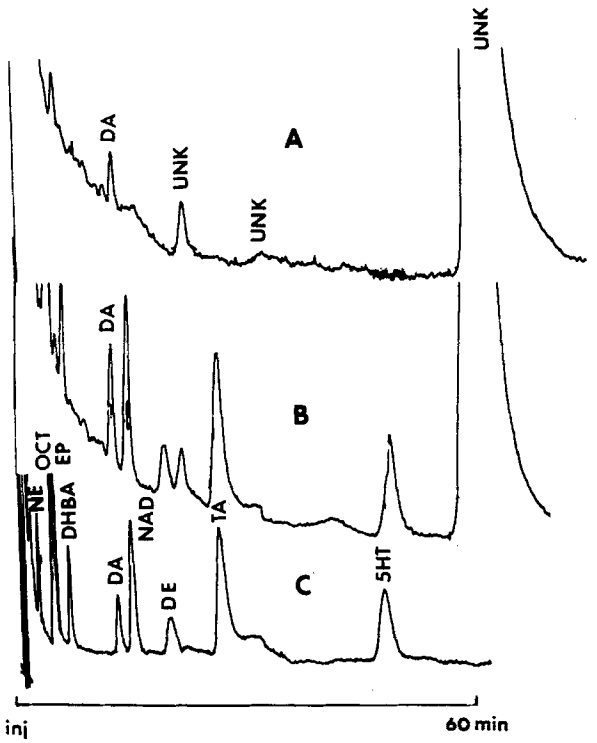

Figure 2. Chromatograms of bulb mite diet (3 g) extract after ion exchange and alumina clean-up. *A* Diet extract only; *B* diet extract plus amine standards; *C* amine standards only. Conditions same as for figure 1.

(fig. 2). The dopamine was found in freshly prepared diet and increased in concentration with increasing age of the diet. The dopamine was in the diet even if the yeast was omitted. However, the presence of dopamine in the diet does not preclude de novo synthesis in the mites. The observation that dopamine was less concentrated in the diet than in the mites suggests that in the latter synthesis is occurring or that a selective uptake mechanism is concentrating dopamine above ambient conditions. These dopamine levels in mites of 4.3 ng g^{-1} (wet wt) are appreciably lower than those reported previously from this mite species in which concentrations of 390 ng g^{-1} for females and 95 ng g^{-1} for males were found[9,10]. Our data represent a mixture of males and females. Also differences existed between the two studies in some aspects of the clean-up and chromatographic techniques. Thus, a plausible reason for the disparity is that the dopamine peak in the earlier work was not homogenous or that the samples were contaminated by diet or both.

Octopamine in mite extracts was identified by cochromatography with the authentic standard (data not shown) and by its response at the two different electrode potentials (fig. 3). Octopamine responds at 0.65 eV at pH 6.0 but not at 0.50 eV.

The data in figure 1 also suggest the presence of *N*-methyldopamine and tyramine, but cochromatography with authentic standards was not obtained under other chromatographic conditions. The low retention times for

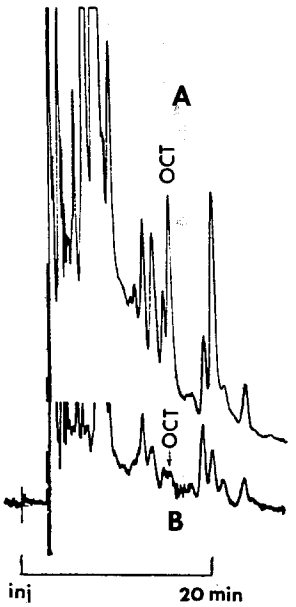

Figure 3. Chromatograms showing the differential response of octopamine at 0.65 eV (A) and 0.50 eV (B) after ion exchange and C_{18} column clean-up of bulb mite (1 g wet wt equivalent) whole body extract. Conditions: 10 µl injection: 0.5 ml min^{-1} flow rate; and 10 nA full scale.

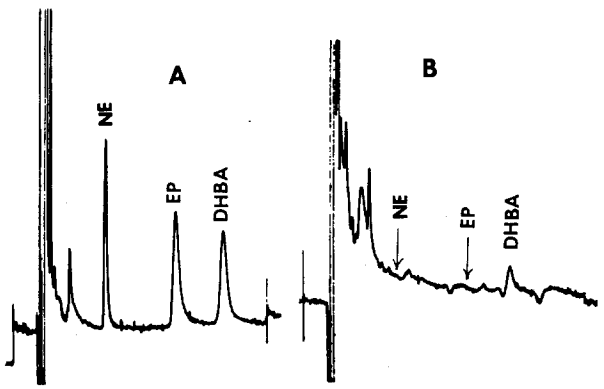

Figure 4. Chromatograms of bulb mite (1 g wet wt equivalent) whole body extract after ion exchange and alumina clean-up under HPLC conditions optimized for resolution of adrenaline (EP) and noradrenaline (NE). *A* Standards only; *B* mite extract only. Conditions: 25 µl injection; 0.5 ml min^{-1} flow rate; no methanol; electrode 0.65 eV; 5 nA full scale.

adrenaline and noradrenaline precluded their successful resolution under the conditions in figures 1 and 2. However, subsequent investigations using slower flow rate and no methanol provided conclusive evidence that these two amines, if present, were below detectable levels (fig. 4). There was no evidence for the presence of *N*-acetyldopamine. 5-Hydroxytryptamine was not detected in mite extracts chromatographed after ion exchange chromatography and before or after the alumina clean-up. Three unknowns were present in the mite extract (fig. 1); the major unknown occurred in the mite diet at higher levels than in the mites (fig. 3). The demonstration of the presence in bulb mites of the biogenic amines dopamine and octopamine represents an important initial step toward our understanding of mite biogenic amine systems.

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