The Biosynthesis of Catecholamines in Two Genera of Protozoa*

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SUMMARY

Norepinephrine was isolated from the flagellated protozoan, Crithidia fasciculata, and the ciliated protozoan, Tetrahymena pyriformis, the former having less than one-half that of the latter. Tetrahymena also synthesizes epinephrine, whereas this substance could not be detected in Crithidia. Isotopically labeled phenylalanine, tyrosine, and dihydroxyphenylalanine were significantly incorporated into the catecholamines in Tetrahymena, while only tyrosine and dihydroxyphenylalanine were incorporated in Crithidia, owing to its lack of a phenylalanine-hydroxylating system.

Catecholamines are hormones which are normally associated with the sympathetic nervous system and have profound physiological effects on smooth muscle. Several recent data, however, suggest additional roles in the central nervous system (1-3). In addition to all groups of vertebrates, these hormones have been found in many invertebrates as well as in a few plants. On the other hand, practically nothing is known of the existence of these hormones in the microorganisms.

The first indication of an involvement of catecholamines in Crithidia fasciculata and Tetrahymena pyriformis was the report by Kidder (4) of a study on the role of unconjugated pteridines in these organisms. In C. fasciculata, a flagellated protozoan that requires tyrosine for growth owing to the lack of a phenylalanine hydroxylase, 3,4-dihydroxyphenylalanine, norepinephrine, and epinephrine significantly reduce the amount of unconjugated pteridine, biotin, required for growth. This indicates the presence of a pteridine-dependent tyrosine hydroxylase that catalyzes the conversion of tyrosine to dihydroxyphenylalanine.

The present study reports the isolation of catecholamines from C. fasciculata and T. pyriformis. After the administration of radioactive precursors, labeled catecholamines were obtained from the cells.

EXPERIMENTAL PROCEDURE

C. fasciculata and T. pyriformis W were grown in the defined media of Kidder and Dutta (5) and Kidder, Dewey, and Hein.

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mug dopamine, 330 and 380 mug norepinephrine, 205 and 505 mug; and epinephrine, 410 and 520 mug.

All the radioactive samples were counted in a Tracerlab ultrathin end window flow counter. The extracts containing the compounds were spread at zero thickness in standard counting planchets, and counting was carried out for a length of time that would ensure reduction of the error to less than 10%.

RESULTS

Table I gives the catecholamine content of Crithidia and Tetrahymena. Dopa and dopamine were not detected even when dopa was used as the substrate. In Tetrahymena, both epinephrine and norepinephrine are present, while norepinephrine is the only catecholamine detected in Crithidia. In Tetrahymena, the amounts of catecholamine are approximately 4 times those in Crithidia.

In Table II are presented data obtained when labeled precursors were used. In Tetrahymena there was a significant incorporation of radioactivity with the three compounds used. In Crithidia there was no incorporation of phenylalanine.

When tyrosine ethyl ester was used as the carrier, the specific activity of the catecholamines obtained from Crithidia was very low, which may be a result of the comparatively higher solubility of or permeability to, the ester as compared to tyrosine. The low specific activity when labeled dopa was used as the substrate may be due to the dilution from tyrosine, which cannot be determined under the conditions of the experiment. When 50 μC of DL-dopa-2-14C were used in 2 liters of culture instead of the usual 1 liter, the specific activity of the catecholamines obtained dropped considerably.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Norepinephrine</th>
<th>Epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. fasciculata</td>
<td>0.1-0.2 µg/ml</td>
<td>0</td>
</tr>
<tr>
<td>T. pyriformis</td>
<td>0.25-0.35 µg/ml</td>
<td>0.13-0.15 µg/ml</td>
</tr>
</tbody>
</table>

### TABLE II

**Incorporation of labeled compounds into catecholamines**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Substrate*</th>
<th>Medium</th>
<th>Specific activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. fasciculata</td>
<td>Tyrosine-14C</td>
<td>Tyrosine ethyl ester, 100 µg/ml</td>
<td>22,600 cpmin/μmole</td>
</tr>
<tr>
<td></td>
<td>Tyrosine-14C</td>
<td>Tyrosine, 100 µg/ml</td>
<td>22,600</td>
</tr>
<tr>
<td></td>
<td>Tyrosine-14C</td>
<td>Tyrosine, 100 µg/ml</td>
<td>37,444</td>
</tr>
<tr>
<td></td>
<td>DL-Dopa-2-14C</td>
<td>Tyrosine, 100 µg/ml</td>
<td>10.6 x 10^4</td>
</tr>
<tr>
<td></td>
<td>DL-Dopa-2-14C</td>
<td>Tyrosine, 100 µg/ml</td>
<td>10.6 x 10^4</td>
</tr>
<tr>
<td></td>
<td>L-Phenylalanine-14C</td>
<td>Tyrosine, 100 µg/ml</td>
<td>6.5 x 10^3</td>
</tr>
<tr>
<td>T. pyriformis</td>
<td>L-Phenylalanine-14C</td>
<td>Phenylalanine, 50 µg/ml</td>
<td>8.2 x 10^4</td>
</tr>
<tr>
<td></td>
<td>L-Tyrosine-14C</td>
<td>Phenylalanine, 50 µg/ml</td>
<td>1.6 x 10^4</td>
</tr>
<tr>
<td></td>
<td>DL-Dopa-2-14C</td>
<td>Phenylalanine, 50 µg/ml</td>
<td>10.6 x 10^4</td>
</tr>
</tbody>
</table>

* All substrates were uniformly labeled except as indicated. In the first line, tyrosine-14C was obtained from Schwarz BioResearch; its specific activity was 300 μC per μmole. The rest of the labeled compounds were obtained from New England Nuclear; their specific activities were: dopa-2-14C, 2.62 μC per μmole; phenylalanine-14C, 375 μC per μmole; tyrosine-14C, 375 μC per μmole.

* Determined by counting an aliquot of freshly inoculated medium.

* As total catecholamines.

* Dopa-2-14C, 50 μC in 2 liters of culture.

Since Crithidia does not have a phenylalanine hydroxylase, one could not expect incorporation of labeled phenylalanine into norepinephrine. The radioactivity obtained initially in the alumina eluates was lost when the extract was chromatographed on Dowex 50, Na⁺ and H⁺ forms, along with authentic samples of catecholamines. This indicates clearly that the radioactivity in the alumina eluates was only a contaminant, possibly attributable to the presence of a metabolite of phenylalanine, possibly phenylethylamine. Further identification of this product was not made.

In Tetrahymena, all the labeled precursors used were incorporated significantly. With dopa-2-14C, the specific activity was much higher than in Crithidia, possibly because of the higher dilution effect in Crithidia caused by the dietary tyrosine. With tyrosine-14C, although the total number of counts incorporated in the catecholamines was higher as compared to the experiments in which phenylalanine was used as the substrate, the low specific activity of the amines isolated, with tyrosine, is due to the dilution from dietary phenylalanine.

It has been shown by Axelrod, Senoh, and Witkop (11) that the major pathway of metabolism of norepinephrine and epinephrine is O-methylation followed by deamination. In the present study we incubated a cell-free extract of Crithidia with 20 μg of norepinephrine and 20 μg of epinephrine. After incubation for 1 hour, the homogenate was adjusted to 0.4 N with perchloric acid and centrifuged. The acid extract was then adjusted to pH 10 with sodium hydroxide and borate buffer (pH 10) and extracted three times with 3 volumes of ethylene dichloride containing 2% isomyl alcohol. The organic phase then was shaken with a small volume of 0.1 N HCl. The acid extract was evaporated in a vacuum, and the residue was taken up in methanol. The methanol extract was concentrated to a small volume and applied as a spot on Whatman No. 1 paper. Authentic samples of normetanephrine, metanephrine, and 3-methoxy-4-hydroxy-mandelic acid were also applied on the paper. The chromatogram was developed for 16 hours in butanol-acetic acid-water (4:1:1). The spots were visualized by spraying with diazotized sulfanilic acid followed by spraying with 20% sodium carbonate. The
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**C. elegans** extract showed two spots corresponding to normetanephrine and 3-methoxy-4-hydroxymandelic acid. No quantitative determination was made. A quantitative determination of the substrate after the termination of incubation showed that norepinephrine decreased considerably but that epinephrine content did not change.

**DISCUSSION**

The catecholamine content of the two protozoans reported here is comparable to the amounts reported in other organisms. The neurohormones have a broad distribution in the multicellular animal and plant world. Brodie, Bogdanski, and Bonomi (12) reported the presence of these amines in lower vertebrates, and their presence in higher vertebrates has long been established. Von Euler (13) reported the occurrence of catecholamines in the invertebrates of the phyla Aschelminthes, Annelida, Arthropoda, Mollusca, and Echinodermata. The amounts reported by him were very small, of the order of 0.003 to 0.005 µg per g (presumably wet weight), except in the larvae of insects, in which the norepinephrine content was found to be 0.33 µg per g. Ferneaux and McFarlane (14) have identified dopa, dopamine, and N-acetyldopamine in the eggs of insects. They suggest a possible relationship between catecholamines and water absorption and think that the amines are involved in some way in the development of the serosal cuticle. So far the only report of the presence of a catechol derivative in microorganisms is by Ostlund (15), who, in the same paper also reported the occurrence of the amine in a few invertebrates. The amine reported by him in the dinoflagellate *Noctiluca* exhibited the same biological activity as norepinephrine. However, the chromatographic behavior of this compound was different from that of norepinephrine, in view of the lack of definite identification, Ostlund named this compound Catechol IV.

The glycogenolytic effect of epinephrine and norepinephrine is well known in mammalian systems. Sutherland and Rall (16, 17) have shown that the primary action of these hormones is on the adenyl cyclase system which effects the production of cyclic 3',5'-AMP, a cofactor in the conversion of phosphorylase b to phosphorylase a, which is the enzyme responsible for the break down of glycogen. Although hormones are not known to control cellular activities in microorganisms, the occurrence of cyclic 3',5'-AMP has been reported in a few bacteria (18, 19). As in mammals, the role of this nucleotide in *Escherichia coli* seems to be regulatory. When the carbon or energy source was limited, Makman and Sutherland (19) noted an abrupt rise in the nucleotide. *E. coli* is known to accumulate some kind of polysaccharide reserve. It is possible, therefore, that the role of the catecholamines in these organisms is to regulate the amounts of polysaccharide reserve in a manner similar to the mammalian systems. Since the primary action of these hormones is on the adenyl cyclase system, the elucidation of the mechanism by which these hormones act upon it is very important for a fuller understanding of the over-all reaction. Another possibility is that the chelating and reducing properties of these hormones have an important bearing on the reaction system.

**REFERENCES**

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