Biosynthesis of Epinephrine and Norepinephrine by Sympathetic Nerves and Ganglia

By McC. Goodall, M.D., Ph.D., and Norman Kirshner, Ph.D

It is generally believed by the neurophysiologists that the sympathetic neurohormone is norepinephrine and not epinephrine as originally supposed. This concept of norepinephrine as the sympathetic neurohormone has evolved from much impressive circumstantial evidence; however, it has not been demonstrated that the biosynthesis of norepinephrine takes place in the sympathetic nerves and ganglia. This paper shows, through the use of labeled precursors, that the sympathetic nerves and ganglia synthesize norepinephrine in a systematic sequence from tyrosine and that this synthesis apparently terminates with norepinephrine rather than progressing to the formation of epinephrine.

The neurohormone of the sympathetic nerves is now generally accepted as being norepinephrine.1-7 Norepinephrine is also found in the adrenal medulla but in most mammals the amount of norepinephrine is considerably less than the amount of epinephrine.3, 8 The neurohormone of the parasympathetic nerves is acetyicholine; however, it appears that many parasympathetic nerves also contain adrenergic fibers.3, 9

Recent evidence indicates that the biosynthetic pathway to epinephrine and norepinephrine formation is via dihydroxyphenylalanine and hydroxytyramine,10, 11 (fig. 1). Goodall and Kirshner11 showed that bovine adrenal slices incubated with tyrosine uniformly labeled with C14 produced in sequence: hydroxytyramine, norepinephrine, and epinephrine. Further, they showed that when C14 dihydroxyphenylalanine (dopa) was incubated with bovine adrenal homogenates, hydroxytyramine, norepinephrine, and epinephrine were synthesized in that order. Gurin and Della

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Hitherto, no one has shown whether sympathetic and parasympathetic nerves are capable of synthesizing epinephrine and norepinephrine. Albeit, Euler 1-2 has shown that the sympathetic nerves, and to a much less degree some parasympathetic nerves, contain small amounts of epinephrine and comparatively large amounts of norepinephrine. Holtz and Westermann14 have shown that the sympathetic and vagus nerves contain dopa decarboxylase. Schäümann15 has demonstrated the presence of hydroxytryramine in extracts of sympathetic nerves, and others6-7 have shown that the sympathetic nerves release norepinephrine.

METHOD

Dogs were anesthetized with Nembutal and killed by bleeding. The splenic nerves, the thoracic sympathetic nerves, the thoracic sympathetic ganglia, and the cervical vagi were immediately removed. In order to compare the result obtained from one species of mammal with that of another, similar nerves and ganglia were obtained from beef immediately after slaughtering.

Approximately 0.10 to 0.20 Gm. of each of these nerve groups were minced with scissors and incubated. A gas mixture of 95 per cent oxygen and 5 per cent carbon dioxide was bubbled through the medium during the incubation period. Each incubation contained 10 μmoles of glucose, 50 μg. of pyridoxal phosphate, 1 μmole of adenosine tri-
phosphate, 0.5 μmole of diphenolphosphoryl nucleotide, and 1 × 10⁴ c.p.m. of uniformly labeled tyrosine with a specific activity of 7.7 μc. per mM, or 4.5 × 10⁴ c.p.m. of dl-dihydroxyphenylalanine-2-C¹⁴ with specific activity of .835 μc. per mM. Extracts of the nerves were then prepared by adding 0.2 volumes of 50 per cent trichloroacetic acid to the incubation medium. The precipitate was removed by filtration and washed with 5 per cent trichloroacetic acid. The filtrate and washings were combined and extracted 3 times with an equal volume of ether to remove the trichloroacetic acid.

In accordance with the procedure described by Kirshner and Goodall, a preliminary purification of the extract was obtained by ion exchange on a 2.5 × 0.9 cm. column of Amberlite IRC-50. The pH of the extract was adjusted to 6.1 to 6.3 and 200 μg, each of nonlabeled hydroxytyramine, norepinephrine, and epinephrine were added as carriers. The extract was placed on the Amberlite column and allowed to run through at a flow rate of approximately 1 ml. per minute. The resin was then washed 3 times with 3-ml. portions of distilled water and the catechol amines were eluted with three 5-ml. portions of 2.0 N acetic acid. The

![Diagram of biosynthetic pathway](image)

**Fig. 1.** Biosynthetic pathway to epinephrine and norepinephrine.

![Graph of optical density vs. tube number](image)

**Fig. 2.** Separation of epinephrine, norepinephrine, and hydroxytyramine from an incubation of sympathetic nerves and C¹⁴-labeled tyrosine. Dotted lines indicate the incorporation of radioactivity in the norepinephrine and hydroxytyramine fractions. From left to right, the peaks represent epinephrine, norepinephrine, and hydroxytyramine. Experimental conditions described under table 2.

<table>
<thead>
<tr>
<th>Type of nerve tissue</th>
<th>Number of experiments</th>
<th>Counts per minute per 0.1 Gm. tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Epinephrine</td>
</tr>
<tr>
<td>Dopa-2-C¹⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenic sympathetic nerve</td>
<td>5</td>
<td>16 ± 4</td>
</tr>
<tr>
<td>Thoracic sympathetic nerve</td>
<td>5</td>
<td>20 ± 20</td>
</tr>
<tr>
<td>Stellate ganglia</td>
<td>5</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>Vagus—cervical</td>
<td>4</td>
<td>5 ± 3</td>
</tr>
<tr>
<td>Boiled sympathetic nerve</td>
<td>4</td>
<td>6 ± 7</td>
</tr>
<tr>
<td>Tyrosine-C¹⁴</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenic sympathetic nerve</td>
<td>4</td>
<td>35 ± 20</td>
</tr>
<tr>
<td>Thoracic sympathetic nerve</td>
<td>4</td>
<td>62 ± 38</td>
</tr>
<tr>
<td>Stellate ganglia</td>
<td>4</td>
<td>41 ± 32</td>
</tr>
<tr>
<td>Vagus—cervical</td>
<td>4</td>
<td>17 ± 19</td>
</tr>
<tr>
<td>Boiled sympathetic nerve</td>
<td>4</td>
<td>28 ± 17</td>
</tr>
</tbody>
</table>

Each incubation contained 1 μmole adenosinetriphosphate, 0.5 μmole of diphenolphosphoryl nucleotide, 10 μmoles of glucose, 50 μg. of pyridoxal phosphate, 2 ml. Kreb’s phosphate buffer¹, pH 7.4, 0.1 to 0.2 Gm. of the minced tissue and either dl-dopa-2-C¹⁴ (4.5 × 10⁴ c.p.m., SA = 0.835 μc./mM) or C¹⁴ uniformly labeled l-tyrosine (8.0 × 10⁴ c.p.m., SA = 7.7 μc./mM). Final volume was 3.0 ml. Incubation time was 3 hours with dopa and 6 hours with tyrosine at 37°.
ammonium acetate and the acetic acid were removed in vacuo at 35°C.

The catechol amines in the extract were then fractionated by column chromatography. The residue was dissolved in 2.0 ml of 0.2 M ammonium acetate buffer pH 6.1. A 1-ml aliquot of the above referred solution was placed on a 30 by 0.9-em. column of Amberlite IRC-50 followed by 1.0 ml of 0.2 M ammonium acetate buffer pH 6.1. The column was then connected to a reservoir of 0.4 M ammonium acetate buffer pH 5.0. The flow rate was adjusted to 3.5 to 4.0 ml per hour; fractions of 1.5 ml were collected.

The amounts of epinephrine, norepinephrine, and hydroxytyramine in each fraction were determined by measuring the optical density at 279 μ. All fractions containing the same catechol amines were pooled and evaporated to dryness in vacuo at 35°C. The residue was dissolved in water and an aliquot plated out for measuring the radioactivity. Radioactivity was measured with a thin window flow counter that had an average background of 16 c.p.m. The data presented have been corrected for background radioactivity.

RESULTS

Dihydroxyphenylalanine (dopa) Incubation

Tables 1 and 2 are tabulations of the radioactivity found in the epinephrine, norepinephrine, and hydroxytyramine fractions after incubating the various nerve groups with C14-labeled dihydroxyphenylalanine (dopa). From these tables, it appears that the sympathetic nerves and ganglia synthesize hydroxytyramine and norepinephrine from dopa. However, the radioactivity in the epinephrine fraction was so low that it was not feasible to purify the material further and thereby to eliminate the possibility of contamination.

The vagal nerves are also capable of synthesizing hydroxytyramine from dopa; however, the radioactivity found in the hydroxytyramine fractions is far less than that found in similar fractions obtained from the sympathetic nerves and ganglia. The vagal nerves do not appear to synthesize epinephrine or any appreciable amounts of norepinephrine.

Tyrosine Incubation

Tables 1 and 2 are tabulations of the radioactivity found in the epinephrine, norepinephrine, and hydroxytyramine fractions after

| Table 2.—Formation of Epinephrine, Norepinephrine and Hydroxytyramine from Dopa-2-C14 and from C14 Uniformly Labeled Tyrosine by Bovine Nerves |
|---------------------------------|-------------------------------|-------------------------------|-------------------------------|
| Type of nerve tissue            | Number of experiments | Counts per minute per 0.1 gm. tissue |
|---------------------------------|------------------------|-------------------------------------|-------------------------------|
| Dopa 2-C14                      |                        | Epinephrine | Norepinephrine | Hydroxytyramine |
| Thoracic sympathetic nerve      | 1                      | 11          | 603            | 16,000           |
| Thoracic sympathetic ganglia    | 1                      | 12          | 317            | 23,360           |
| Stellate ganglia                |                        |            |                |                  |
| 3 hr. incubation                | 1                      | 29          | 332            | 39,400           |
| 6 hr. incubation                | 2                      | 8 ± 2       | 140 ± 25      | 590 ± 476        |
| Cervical sympathetic nerve      | 1                      | 8           | 27             | 900              |
| Vagus—cervical                  | 1                      | 4           | 9              | 500              |
| Tyrosine C14                    |                        |            |                |                  |
| Thoracic sympathetic nerve      | 3                      | 16 ± 7      | 417 ± 158      | 73 ± 31          |
| Thoracic sympathetic ganglia    | 3                      | 13 ± 7      | 653 ± 270      | 94 ± 23          |
| Stellate ganglia                |                        |            |                |                  |
| 6 hr. incubation                | 3                      | 14 ± 7      | 763 ± 458      | 107 ± 48         |
| Cervical sympathetic nerve      | 1                      | 6           | 70             | 33               |
| Vagus—Cervical                  | 1                      | 10 ± 3      | 12 ± 1         | 4 ± 1            |
| Boiled sympathetic nerve        | 2                      | 2 ± 3       | 4 ± 1          | 2 ± 1            |

Each incubation contained 1 μmole of adenosine triphosphate, 0.5 μmole of diphosphopyridine nucleotide, 10 μmoles of glucose, 50 μg of pyridoxal phosphate, 2 ml of Kreb's phosphate buffer pH 7.4, 0.1 to 0.2 gm. of the minced tissue and either dl-dopa-2-C14 (4.5 x 105 c.p.m., SA=0.835 mc./mM), or C14 uniformly labeled L-tyrosine (7.8x105 c.p.m., SA=16.3 mc./mM). Final volume was 3.0 ml. Incubation time was 3 hours with dopa and 6 hours with tyrosine at 37°C.
incubating the various nerves and ganglia with uniformly labeled tyrosine. From these tables it appears that the sympathetic nerves and ganglia synthesize hydroxytyramine and norepinephrine; however, from this data it is again impossible to conclude whether or not epinephrine is formed. Figure 2 presents the results obtained from incubating bovine thoracic sympathetic chain with labeled tyrosine. Here the radioactivity and the optical density are plotted against the fraction numbers.

The radioactivity of the hydroxytyramine fraction formed by incubating dopa with sympathetic nerves and ganglia was greater than the radioactivity of the hydroxytyramine fraction formed by incubating with tyrosine. This is because the conversion from dopa to hydroxytyramine is rapid, while that from tyrosine to dopa is slow.

The vagal nerves appear incapable of synthesizing epinephrine, norepinephrine or hydroxytyramine from tyrosine. This would then lead one to believe that cholinergic nerves do not normally synthesize norepinephrine.

General Results

The results presented in tables 1 and 2 are calculated on the basis of counts per minute per 0.1 Gm. of tissue; however, from the specific activity of the tyrosine and dopa, and from the amount of radioactivity in the nor-epinephrine fraction, the absolute amounts of norepinephrine found after the incubations have been calculated. With dog sympathetic nerves and ganglia, the average total amount found after a 3-hour incubation with dopa was 0.51 ± 0.39 μg; after a 6-hour incubation with tyrosine the average total amount found was 0.072 ± 0.066 μg. With bovine sympathetic nerves the average total amount found after a 3-hour incubation with dopa was 1.08 μg, and after a 6-hour incubation with tyrosine the amount found was 0.078 ± 0.036 μg. The amounts of norepinephrine found are the differences between the amounts synthesized and the amounts destroyed by monoamine oxidase, possibly other enzymes and by chemical oxidation. That this destruction is considerable can be seen from table 2, in which the amounts of nor-epinephrine found after a 6-hour incubation of the stellate ganglia with dopa is less than one half the amount found after a similar 3-hour incubation. Previous work on the formation of epinephrine and norepinephrine from tyrosine in the adrenal medulla indicated that maximum norepinephrine formation was found after a 6-hour incubation.

Dopamine and Tyrosine Incubated with Marsilid

Sympathetic nerves were incubated with dopa and Marsilid. Results of these experiments showed an increase in the radioactivity of the hydroxytyramine fraction. In that Marsilid is a monoamine oxidase inhibitor, it inhibits oxidation of hydroxytyramine and thereby apparently permits hydroxytyramine to accumulate. This is less apparent when it is incubated with tyrosine, since the conversion from tyrosine to dopa is slow.

Discussion

Epinephrine has long been known to be present in the adrenal gland and for many years was thought to be the neurotransmitter of the sympathetic nerves. However, as early as 1910 Barger and Dale questioned the validity of epinephrine as the sympathetic neurotransmitter. In 1946 Euler showed that the sympathetic nerves contained predominantly norepinephrine. Recent work supports Euler's concept of norepinephrine as the sympathetic neurotransmitter.

Hitherto, there has been little evidence indicating the biosynthetic pathway of norepinephrine formation in adrenergic nerves, although it would seem that the pathway should be identical with that in the adrenal gland. Experiments herein described show that the sympathetic nerves and ganglia incubated either with tyrosine or dopa synthesized hydroxytyramine and norepinephrine, but only questionable amounts of epinephrine. Therefore, it appears that the same biosynthetic pathway does occur in the sympathetic nerves and ganglia as in the adrenal medulla (fig.
1). However, since the amount of epinephrine formed by the nerves was hardly detectable, one could only conclude that if epinephrine is synthesized, the rate of synthesis is significantly slow. In contrast the norepinephrine was readily detected and significantly high. Therefore, it seems reasonable that the neurotransmitter of the sympathetic nerves is norepinephrine, and that epinephrine, if present, is of questionable importance in sympathetic nerve transmission.

Also from the experiments herein described, it appears that the vagus nerve is incapable of synthesizing epinephrine or norepinephrine from tyrosine or dopa. However, some slight radioactivity noted in the hydroxytyramine fraction after incubation with dopa, would indicate that the vagus nerve could synthesize hydroxytyramine. Euler2,3,20 showed that the vagus nerve contained small but definite amounts of norepinephrine and found that this was highly suggestive of the presence of adrenergic fibers within the vagus. The absence of radioactive norepinephrine in the vagus nerve incubation experiments probably means that the amount of radioactive norepinephrine formed was so small that its detection was inconclusive. Therefore, it would seem that cholinergic nerves do not synthesize epinephrine or norepinephrine, but if they do, the quantity formed is significantly small.

SUMMARY

With the use of labeled precursors, the biosynthesis of epinephrine and norepinephrine by sympathetic ganglia and nerves and parasympathetic nerves was studied. Sympathetic nerves and ganglia incubated with either labeled tyrosine or labeled dihydroxyphenylalanine formed radioactive hydroxytyramine and norepinephrine. The vagus nerve incubated with either labeled tyrosine or labeled dihydroxyphenylalanine formed no significant amounts of radioactive epinephrine or norepinephrine and only small amounts of radioactive hydroxytyramine. The evidence presented supports the concept that norepinephrine is the neurotransmitter of the sympathetic nerve.

SUMMARIO IN INTERLINGUA

Per medio del marceation del precursores de epinephrina e norepinephrina, le biosynthese de iste substantia in gangliones e nervos sympathetic e in nervos parasympathetic esseva studiate. Nervos e ganglions sympathetic incubate con tyrosina marcate o con dihydroxyphenylalanina marcate formava radioactive hydroxytyramina e norepinephrina. Le nervo vage incubate con tyrosina marcate o con dihydroxyphenylalanina non formava significative quantitates de radioactive epinephrina o norepinephrina e solmente parve quantitates de radioactive hydroxytyramina. Es supportate le conception que norepinephrina es le neurohormon del nervo sympathetic.

REFERENCES

BIOSYNTHESIS OF EPINEPHRINE AND NOREPINEPHRINE


The 8 patients described in this paper have 3 features in common: all of them developed shock in the terminal phases of their illnesses, all 8 were treated vigorously with levarterenol (Levophed), and diffuse damage involving the proximal tubules associated with changes in the glomeruli and blood vessels were present. These lesions in the kidney are similar to the "proximal tubular necrosis" with "focal cortical necrosis" described by Sheehan and Moore and attributed by them to vasospasm. It is therefore suggested that the postmortem findings with respect to the kidneys, in the 8 patients described in this report, are the result of severe ischemia due to spasm of the renal arterioles and the medullary veins. The cause of the spasm is believed to be shock which was intensified and prolonged by the therapy with levarterenol.

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