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METABOLISM OF 3,4-DIHYDROXYPHENYLALANINE (L-DOPA) IN HUMAN SUBJECTS*

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Abstract—Three healthy males were infused with 100 μc 3,4-dihydroxyphenylalanine (L-dopa)-3-^{14}C in 1000 ml of physiological saline. Urine was collected during the infusion period, at 2, 4, 8 and 24 hr, and daily thereafter for 5 days. Using a specially designed flow monitor system, the various L-dopa metabolites and biosynthetic products were separated, identified, and their radioactivity measured. Of the total radioactivity infused, 71·6 $\pm$ 3·1 per cent was recovered in 24 hr and 80·6 $\pm$ 3·4 per cent in 120 hr. Thirty-five radioactive metabolic products were separated. Of the infused L-dopa-3-^{14}C, 11 per cent of the radioactivity was recovered as metabolic products of L-dopa, 64 per cent as dopamine or metabolic products of dopamine, and 5 per cent as noradrenaline or metabolic products of noradrenaline; no radioactive adrenaline was detected. The remaining 20 per cent of radioactivity was unaccounted for and was presumably distributed and subsequently released. Of the 35 metabolic products which were separated, 16 were identified, 2 were tentatively identified and 17 were unidentified. The identified products represented 65·7 per cent of the total radioactivity recovered in 120 hr.

DL-(3,4-DIHYDROXYPHENYLALANINE) (DL-dopa) was first synthesized by Funk in 1911,[1] at which time he postulated its role in the synthesis of adrenaline. However, L-dopa was not found to occur naturally in mammalian tissue until 1950, at which time Goodall[2,3] demonstrated its presence along with dopamine in the adrenal medulla. Whereas there is indirect evidence that L-dopa occurs in other neurogenic tissue, it nevertheless has not been isolated from these tissues. This, however, is understandable in that these same tissues are also abundantly rich in the enzyme L-dopa decarboxylase,[4–10] which rapidly decarboxylates dopa to dopamine (3,4-dihydroxyphenylethylamine, 3-hydroxytyramine).

There are areas of the brain which, in addition to containing noradrenaline (norepinephrine), contain relatively large amounts of dopamine or dopaminergic neurons, i.e. corpus striatum, hypothalamus, substantia nigra.[11–23] Since dopa is the immediate precursor to dopamine,[24–26] it is therefore reasonable to assume that these neurons contain L-dopa even if the dopa has not been directly identified. In patients with idiopathic Parkinsonism, the corpus striatum contains subnormal quantities of dopamine[18–21] and this deficit, along with an alteration in dopamine metabolism and a decrease in noradrenaline synthesis,[27] is believed to be related to the pathogenesis of this syndrome. Whereas an infusion of dopamine in Parkinsonism patients at first appeared to be the logical approach to treating this depletion, nevertheless this did not prove successful, since the corpus striatum was relatively impermeable to

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circulating dopamine. Therefore, L-dopa, which does enter into the cells of the corpus striatum, has been used successfully in the treatment of Parkinsonism;[28–31] evidence is that the intraneural L-dopa is converted to dopamine and noradrenaline via the same pathways by which it is converted by the sympathetic nerves and ganglia,[26] but in the caudate nucleus and in dopaminergic neurons the conversion is more restricted to the formation of dopamine.[32,33] In view of the very limited understanding of the metabolism of L-dopa and the current clinical importance of this compound in the treatment of Parkinsonism, it seems pertinent to evaluate the normal metabolism of circulating L-dopa in the human.

METHODS

Infusion of L-*dopa*-3-^{14}C *and collections of post-infusion urine*

General. Three normal, healthy males (ages 21–35) were infused with 100 μc L-dopa-3-^{14}C. The labeled L-dopa was dissolved in 1000 ml of physiological saline and infused into the antecubital vein at a constant rate over a period of 4 hr. The subjects were kept supine during the infusion and thereafter maintained in a sedentary state until the termination of the collection periods. Urine was collected during the infusion period and at 2-hr intervals for the first 4 hr post-infusion, then at the end of 8, 24 hr, and at the end of the second, third, fourth and fifth days. The urine samples were immediately frozen and stored at −20° until assayed. The infused L-dopa-3-^{14}C was chromatographed and found to be more than 97·5 per cent pure.

Isolation and quantitation of metabolic products of L-*dopa*

Separation of the acidic metabolites and their conjugates. An aliquot of urine containing 100,000 dis/min along with carrier compounds of 3-methoxy-4-hydroxymandelic acid (MOMA), 3-methoxy-4-hydroxyphenylacetic acid (HVA), 3,4-dihydroxy mandelic acid (DOMA) and 3,4-dihydroxyphenylacetic acid (DOPAC), was placed on a 1 × 35 cm column of Dowex-1-X2 acetate anion-exchange resin. The column was attached to a specially designed flow–monitoring system. In this system, the column was connected to an autogradient elution system and eluted with water followed by a variable gradient elution consisting of ammonium acetate buffers of varying molarity and acidity. The elution was carried out in four steps: Elution A was composed of two series-connected chambers each containing 500 ml of solution; in the first was water, in the second was 0·02 M, pH 4·0 ammonium acetate buffer. Elution B was composed of four series-connected cylinders, each of which contained 300 ml of solution; the first and third contained water, the second contained 1·5 M, pH 4·6 ammonium acetate buffer, and the fourth 6·0 M, pH 4·6, ammonium acetate buffer. Elution C was composed of 500 ml of 6·0 M, pH 4·8, ammonium acetate buffer, and elution D was 500 ml of 6·0 M, pH 6·0, ammonium acetate buffer.

In this flow-monitoring system, the column eluate first passed through an ultraviolet spectrophotometer where the optical density was measured and recorded on one channel of a dual recorder. From the ultraviolet spectrophotometer, the eluate then passed into a scintillation counter with a 10-ml flow cell where the radioactivity was monitored and recorded on the second channel of a dual recorder. The scintillation counter was instructed by a volume-measuring device to indicate digitally the total counts accumulated during each present volume collection and to punch tape the

same information for computer use. From the scintillation counter, the eluate then passed to a fraction collector where the various fractions were collected and recorded. In this manner, continuous intergration was performed during the course of the column elution. A typical elution pattern recording obtained with this system is shown in Fig. 1. The punch tape is fed into a NOVA 8 K computer which is programmed to print out the percentage of radioactivity of each peak (metabolite) in terms of the amount infused and in terms of each collection period, i.e. 0–2, 2–4 hr, etc. (see Table 1).

The recovery of the total radioactivity placed on the Dowex-1 column was 97 $\pm$ 6 per cent. The peaks containing specific free phenolic acids, such as MOMA, HVA, DOMA and DOPAC, were confirmed by paper chromatography of each peak in three solvent systems: *n*-butanol–1 N acetic acid–H_2O (4:1:1); benzene–propionic acid–H_2O (8:2:2) and isopropanol–5% NH_3 (8:2). In order to chromatograph these peaks, it was necessary first to remove the ammonium acetate. The conjugates of HVA, DOPAC and MOMA were identified by refluxing the ammonium acetate free peak in 3 N H_2SO_4 followed by extraction into ether and chromatography of the resulting compound with appropriate carrier compounds in the solvent system described above. The conjugates of 3-methoxytyramine and 3-hydroxytyramine (dopamine) were identified by hydrolysis of the conjugated amine in 2 N H_2SO_4 followed by ion-exchange absorption and chromatography of the free amines.

Separation of the basic metabolites. An aliquot of urine containing 500,000 dis/min was placed on a 30 $\times$ 0·9 cm column of Dowex 50-X4 resin along with the carrier compounds phenylalanine, tyrosine, 3-methoxytyrosine, normetadrenaline, noradrenaline, 3-hydroxytyramine (dopamine) and 3-methoxytyramine. As described above under "Separation of the acidic metabolites and their conjugates", the basic metabolites were all similarly separated and their radioactivity was monitored, but the resin and the elution were different. The basic metabolites were eluted with ammonium acetate of varying molarity and pH. The elution was carried out in two steps. Elution A was composed of four series-connected chambers, each of which contained 300 ml of solution; the first and second contained water, and the third and fourth contained 0·5 M, pH 3·8 ammonium acetate buffer. Elution B was made up of three series-connected cylinders, each containing 300 ml of solution; the first and second contained water, and the third contained 6·0 M pH 4·8, ammonium acetate buffer. The large radioactive peak at the beginning of the elution contained the acidic metabolites. Figure 2 represents the typical elution obtained with this system. The identity of the various radioactive peaks was determined and confirmed by paper chromatography.

RESULTS

The total amount of radioactivity recovered in 24 hr after an infusion of 100 μc L-dopa-3-^{14}C was 71·6 $\pm$ 3·1 per cent and in 120 hr, 80·6 $\pm$ 3·4 per cent. Of the infused L-dopa, approximately 11 per cent of the radioactivity was recovered as metabolic products of dopa, 64 per cent as dopamine or metabolic products of dopamine, and 5 per cent as noradrenaline or metabolic products of noradrenaline; no adrenaline was detected (Fig. 3). The remaining 20 per cent was presumably stored and subsequently released. Thirty-five radioactive products were separated from the urine and of these 16 were identified, 2 were tentatively identified and the rest were unidentified.

The identified products represented 65·7 per cent of the total radioactivity recovered in 120 hr. Most of the identified products were either biosynthetic products of dopamine, i.e. noradrenaline and metabolic products of noradrenaline, or the metabolic products of dopamine of which DOPAC and HVA were the principal ones.

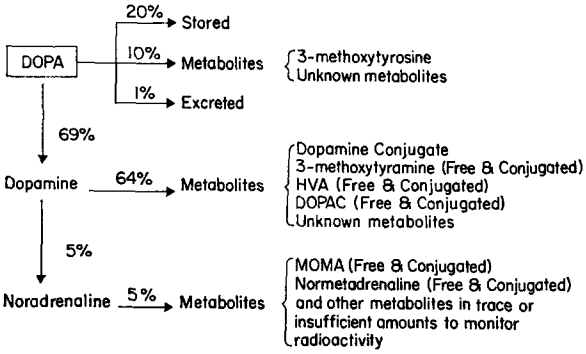

FIG. 3. Distribution of intravenously infused L-dopa-3-^{14}C and its metabolites at 120 hr post-infusion.

Of the total amount of radioactivity recovered in 120 hr, 18·0 ± 6·1 per cent was HVA and 8·6 ± 3·4 per cent was DOPAC (Table 1 and Fig. 4). Other dopamine metabolic products were 3-methoxytyramine, 3,4-dihydroxyphenylethanol, 3-methoxy-4-hydroxyphenylethanol (MHPE) and conjugates of dopamine, MHPE (peak 19), HVA and DOPAC, plus several unidentified metabolic products, i.e. acidic peaks 9, 16, 18 and 23. The remaining unidentified metabolic products listed in Table 1 represent biosynthetic or metabolic products of DOPA *per se* rather than products of dopamine or noradrenaline. Peak 2 (acidic) appears to contain 3,4-dihydroxyphenylethanol and MHPE.

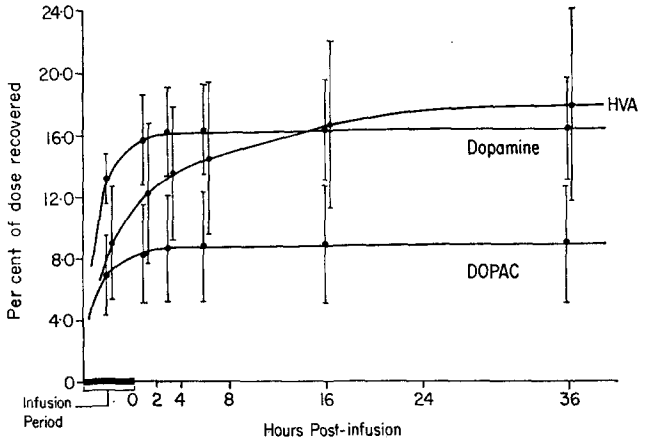

FIG. 4. Accumulative recovery of the infused radioactivity as 3-methoxy-4-hydroxyphenylacetic acid (HVA, homovanillic acid), dopamine (3-hydroxytyramine) and 3,4-dihydroxyphenylacetic acid (DOPAC) from the beginning of the infusion of L-dopa-3-^{14}C for 36 hr post-infusion.

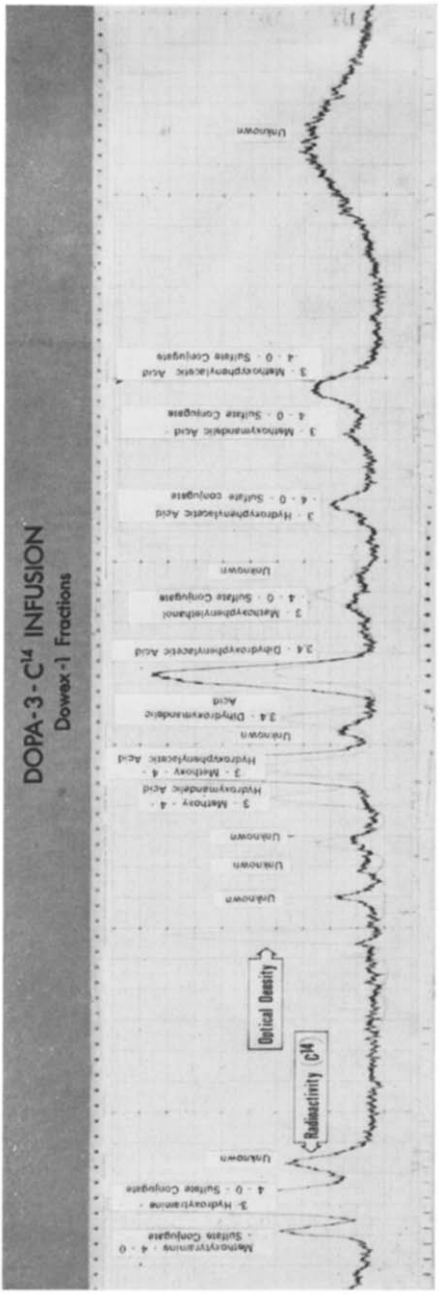

FIG. 1. Representative tracing of the acidic metabolites and conjugates of L-dopa-3-^{14}C as they are eluted from the Dowex-1-X2 column.

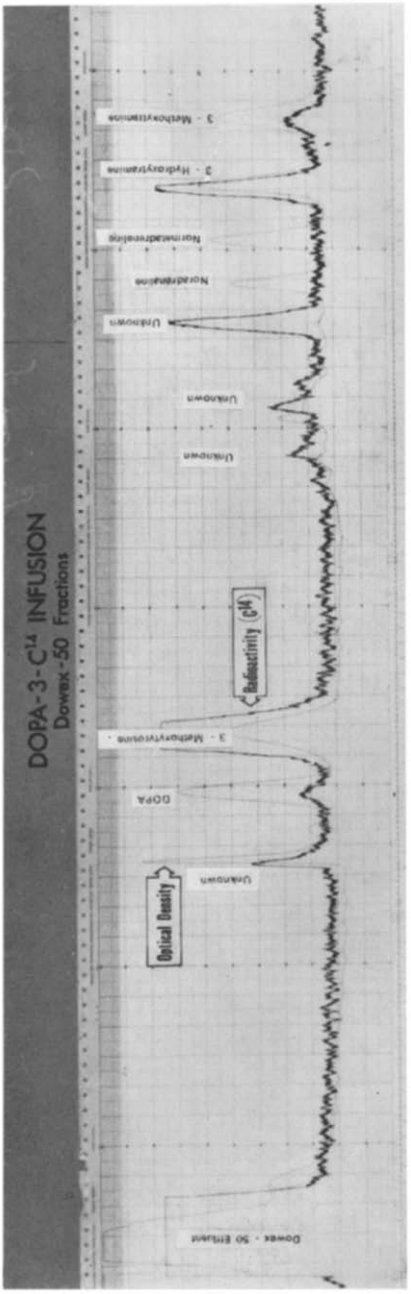

FIG. 2. Representative tracing of the basic metabolites of L-dopa-3-^{14}C as they are eluted from the Dowex 50-X4 column.

The largest amount of radioactive dopa was recovered during the infusion period and represented 1·03 ± 0·13 per cent of the total radioactivity infused. There was a rapid decrease in the amount of radioactive dopa such that only trace amounts were recovered in the 8–24 hr collection period and none thereafter (Table 1 and Fig. 5). Concomitant with this decrease in dopa was an increase in 3-methoxytyrosine (3-M-tyrosine), the principle metabolite of dopa (Fig. 5).

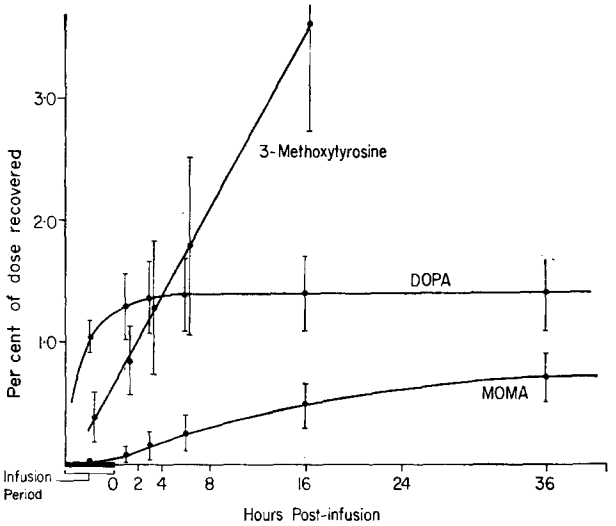

FIG. 5. Accumulative recovery of the infused radioactivity as L-dopa, 3-methoxytyrosine and 3-methoxy-4-hydroxymandelic acid (MOMA) from the beginning of the infusion of L-dopa-3-^{14}C for 36 hr post-infusion.

Approximately 5 per cent of the dopamine synthesized from the L-dopa-3-^{14}C was further synthesized into noradrenaline, as reflected by the recovery of trace amounts of noradrenaline and normetadrenaline but relatively larger amounts of MOMA and the conjugates of MOMA (Table 1 and Fig. 5). Whereas there were only trace amounts of radioactive MOMA recovered in the infusion period, this gradually increased such that in 120 hr 0·7 ± 0·2 per cent was recovered as MOMA and 1·2 ± 0·9 per cent as the conjugate of MOMA.

There are a large number of radioactive products which appear to be the biosynthetic or metabolic products of L-dopa rather than of dopamine or noradrenaline. This is supported by the fact that with previous similar infusion experiments using labeled dopamine,[34] noradrenaline[35] or adrenaline,[36,37] these dopa-related products did not appear in the urine. The dopa-related products represent approximately 11·0 per cent of the total amount of radioactivity recovered in 120 hr. They are represented by peaks 4B, 7B, 8B, 9B and 10B of the basic elution and by peaks 5, 6, 7, 8, 10 and 14 of the acidic elution (Table 1); most of these have not yet been identified.

Of particular interest is peak 4 (basic) which contains two closely eluted compounds that in this system elute as tyrosine and phenylalanine. This peak represents 0·4 ± 0·1 per cent of the infused radioactivity. The greatest amount of these compounds

was recovered during the infusion period (Table 1). Consideration was given to the possibility that these two compounds were contaminants of the infused L-dopa, but chromatographic studies of the L-dopa-3-^{14}C indicated otherwise.

The conjugate of dopamine (3-HT conj.) was recovered in the greatest amount during the infusion period, i.e. 2·0 $\pm$ 1·0 per cent of the infused dose, and gradually decreased in each subsequent collection period. The recovery parallels the recovery of dopamine. The total amount of dopamine conjugate recovered in 120 hr was 6·4 $\pm$ 2·2 per cent of the infused L-dopa. Peak 19 (acidic) has been tentatively identified as the conjugate of 3-methoxy-4-hydroxyphenylethanol (MHPE conj.), which is a metabolic product of dopamine; it represents 1·3 $\pm$ 0·6 per cent of the infused L-dopa. Acidic peaks 9, 16, 18 and 23 are also metabolic products of dopamine and have not yet been identified;[34] they represent about 5·5 per cent of the total radioactivity recovered in 120 hr post-infusion.

DISCUSSION

DL-Dopa was first synthesized in 1911. Previously and subsequently, several investigators postulated the role of tyrosine[38–40] and, considerably later, the role of dopa[24] in the synthesis of adrenaline. However, it was not until 1950 that L-dopa along with dopamine was shown to occur naturally in mammalian tissue.[2,3] L-dopa is the immediate precursor to dopamine,[24–26] and is currently being used in the treatment of idiopathic Parkinsonism.[28–31]

From the L-dopa-3-^{14}C infusion experiments herein described, 80·6 $\pm$ 3·4 of the radioactivity was recovered in the urine in 120 hr. Of the infused (circulating) L-dopa, 11 per cent was metabolized directly into metabolic products of dopa of which 3-methoxytyrosine is the principal metabolite. Approximately 64 per cent was synthesized into dopamine and metabolic products of dopamine. Another 5 per cent was synthesized into noradrenaline via dopamine and appeared in the urine as noradrenaline or metabolic products of noradrenaline (Fig. 3 and Table 1). The remaining 20 per cent of the infused radioactivity is unaccounted for and presumably is distributed throughout the dopaminergic–sympathetic nervous system, in the epidermis as melanin, and in other tissues. There were no detectable amounts of radioactive adrenaline or direct metabolic products of adrenaline.

Since approximately 69 per cent of the labeled L-dopa was converted to dopamine and noradrenaline, this would mean that the circulating L-dopa was taken up by various tissues including the kidney, liver, heart, dopaminergic neurons, sympathetic system, and decarboxylated to dopamine. In those tissues in which dopamine is the principal neurohormone, i.e. dopaminergic neurons and related basal ganglia, the dopamine is presumably released as such and is subsequently metabolized. However, the amount of L-dopa-3-^{14}C utilized by dopaminergic neurons, at most, would represent a very small percentage of the amount infused.

Some of the infused (circulating) L-dopa-3-^{14}C is directly taken up by the sympathetic nervous system. However, most of it, approximately 65–70 per cent, is decarboxylated during the circulation to dopamine. Of the resulting circulating dopamine, 25 per cent is synthesized (aliphatic hydroxylation) into endogenous noradrenaline.[34] The tissue principally involved in this noradrenaline synthesis is the sympathetic nervous system.[11,26]. The noradrenaline released from the sympathetic nerves is for the most part deaminated to 3,4-dihydroxymandelic acid (DOMA)[35,36,41] and then

O-methylated to MOMA,[35,42] but other related noradrenaline metabolic products[35] are also formed and appear in the urine (Table 1). The largest single metabolic product of noradrenaline is MOMA and in these L-dopa infusion experiments there is a gradual increase in MOMA from the infusion period to the 24–48 hr collection period (Table 1 and Fig. 5). When these latter results are extrapolated in terms of conversion of noradrenaline to MOMA, it appears that about 5 per cent of the infused L-dopa is ultimately synthesized into noradrenaline.

The remaining (circulating) dopamine formed from L-dopa-3-^{14}C, i.e. that which is not incorporated in the sympathetic system or the dopaminergic system, is for the most part metabolized into dopamine metabolic products. This is attested to by the rapid decrease in the recovery of radioactive dopamine during the infusion period and the concomitant increase in the recovery of radioactive dopamine metabolic products, of which there are 12. DOPAC, HVA and their respective conjugates are the principal dopamine metabolites; in terms of radioactivity, DOPAC represents 8·6 $\pm$ 3·4 per cent in 120 hr and HVA represents 18·0 $\pm$ 6·1 per cent. The DOPAC formed is rapidly *O*-methylated to HVA or conjugated, which explains the rapid decrease in DOPAC from the infusion period (Table 1 and Fig. 4).

The question naturally arises as to why such a large percentage of the infused L-dopa-3-^{14}C is so rapidly converted to dopamine. The most reasonable explanation resides in the ubiquity and activity of the enzyme dopa decarboxylase.[43–45] Since dopa decarboxylase is found in most tissues and since it rapidly decarboxylates dopa to dopamine, it then follows that the infused L-dopa-3-^{14}C would be systematically exposed to decarboxylation and consequently dopamine would be rapidly formed. These experiments demonstrate the rapidity with which L-dopa-3-^{14}C is decarboxylated, i.e. 1·03 $\pm$ 0·13 per cent in the infusion period, and the concomitant appearance of large amounts of radioactive dopamine (13·2 $\pm$ 1·8 per cent) and dopamine metabolites (Table 1, Figs. 4 and 5). Whereas the enzyme dopa-oxidase also acts upon L-dopa, it nevertheless is more restricted to the synthesis of melanin.

The recovery of radioactivity in peak 4B, tentatively identified as containing phenylalanine and tyrosine, is of particular interest since it implies that the step from tyrosine to L-dopa is a reversible one. This makes even more meaningful Blaschko's[46] earlier finding that dopamine-to-noradrenaline was a reversible reaction and that dopamine was not only a precursor of noradrenaline but also a metabolite.

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