Biochemical Pharmacology, 1963, Vol. 12, pp. 265–269. Pergamon Press Ltd., Printed in Great Britain.

DISTRIBUTION AND FATE OF DIHYDROXYPHENYLALANINE-2-^{14}C (DOPA) IN MICE

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(*Received* 19 *October* 1962; *accepted* 1 *November* 1962)

Abstract—The distribution in whole mice of intravenously injected dihydroxyphenylalanine-2-^{14}C (dopa) has been studied with an autoradiographic technique.

The radioactivity initially accumulated in the pancreas and other organs characterized by a rapid protein synthesis, and also in the adrenal medulla.

Later on, the radioactivity vanished from most tissues but increased in the adrenal medulla. After 4 days the adrenal medulla was the only site where radioactivity was observed.

Separation by paper chromatography revealed that in the pancreas most of the activity was related to dopa. A certain amount was also found in the dopamine fraction. In the adrenal medulla, labelled dopamine and noradrenaline could be identified 30 min after administration Four days after the injection of ^{14}C-dopa, the radioactivity in the adrenal medulla was found to represent mostly adrenaline but also to some extent noradrenaline.

INTRODUCTION

THE biosynthesis of catecholamines seems to proceed along the pathway suggested by Blaschko.[1] By introduction of a phenolic OH group into tyrosine, dihydroxyphenylalanine (dopa) is formed which, in turn, is decarboxylated to dopamine. Dopamine is then hydroxylated to noradrenaline which is finally methylated to adrenaline. By means of labelled catecholamine precursors, Udenfriend and Wyngaarden[2] found direct evidence for this sequence of synthesis in the adrenal medulla of rat.

Although the pathway for the biosynthesis is fairly well established, the location of some of the reactions involved seems to deserve further consideration. Thus, dopa itself has not been identified in any organ under ordinary conditions. On the other hand, many organ extracts including adrenal medulla and sympathetic nerves,[3, 4] have the ability to decarboxylate dopa to dopamine. It is therefore reasonable to suggest that the conversion of dopa to dopamine occurs in the adrenal medulla and in sympathetic nerves. However, the enzyme responsible for the decarboxylation of dopa has been found to be nonspecific. A number of amino acids are decarboxylated by the same enzyme.[5] Thus, the occurrence of a decarboxylating enzyme does not allow conclusions regarding the location of the conversion of dopa to dopamine.

Earlier investigations with slices from adrenal medulla and homogenates from sympathetic nerves have shown that these tissues have the ability to convert ^{14}C-dopa to catecholamines.[6, 7] However, these *in vitro* studies gave only a small quantitative yield and it is still an open question where the major conversion of dopa to catecholamines takes place.

The present study deals with the distribution of ^{14}C-dopa in whole mice. The change in distribution at intervals after the administration of dopa was followed. The substance used was labelled in 2-position. Therefore, not only dopa, but also the decarboxylated metabolites, were labelled. The distribution was followed by an autoradiographic technique.[8] In some organs, the radioactive material was separated by means of paper chromatography.

By determining the distribution of dopa, it was hoped to gain further information regarding the biosynthesis of catecholamines. Dopa has also attracted our interest as a physiologic amino acid analogue.

METHODS

A. Autoradiography

The labelled dopa, DL-3(3:4-dihydroxyphenyl)-alanine-2-^{14}C, was obtained from the Radiochemical Centre, Amersham, England. The specific activity was 0·835 mc/mmole.

The substance was dissolved in physiological saline and 0·2 ml solution was injected intravenously into mice. The dose was about 0·3 mg ^{14}C-dopa per animal.

White adult mice, weighing about 20 g, were used as experimental animals. In one series, consisting of seven mice, the animals were killed at the following intervals after injection: 5 min, 20 min, 40 min, 1 hr, 4 hr, 24 hr and 4 days. A pregnant mouse was also injected and killed after 30 min.

The mice were anaesthetized with ether and killed by immersion in a mixture of acetone and solid carbon dioxide at about −70 °C. Sagittal 20 μ sections through the whole frozen animals were cut and dried at −10 °C.

Autoradiographic exposure was made by apposition against Gevaert Dentus Rapid film. The exposure time was about 10 days. The autoradiographic method has been described in detail by Ullberg.[8]

B. Chromatography

The paper chromatographic investigation was planned with guidance from the autoradiographic results. As in the autoradiographic experiments, white mice (weighing 20 g) were employed. Each animal received 0·25 μc per g body weight.

The tissues examined for radioactive components were adrenals and pancreas. Samples from these organs were taken 10 min and 20 min after injection. Adrenals were also investigated from mice killed 4 days after injection. In order to obtain enough adrenal tissue material for colour reactions the tissue specimens from ten animals were pooled.

The organs were homogenized in acid ethanol (1 ml conc. HCl/1000 ml ethanol). After filtration, the extracts were evaporated to dryness *in vacuo*. The residue was taken up in a few millilitres of 0·01 N HCl and shaken with water saturated ether to remove lipids. After evaporation almost to dryness, aliquots of the extract were placed on paper (Whatman no. 1). Reference substances were added to extracts from organs of mice not injected with dopa. The chromatograms were run in phenol–0·1 N HCl (85:15, w/v) for 30 hr or in *n*-butanol–1 N HCl (4:1) for 24 hr. The spots were developed by spraying with 0·44% $K_3Fe(CN)_6$. The radioactivity on the chromatograms was detected by pressing the chromatograms against Kodak no screen X-ray film.

facing page 266

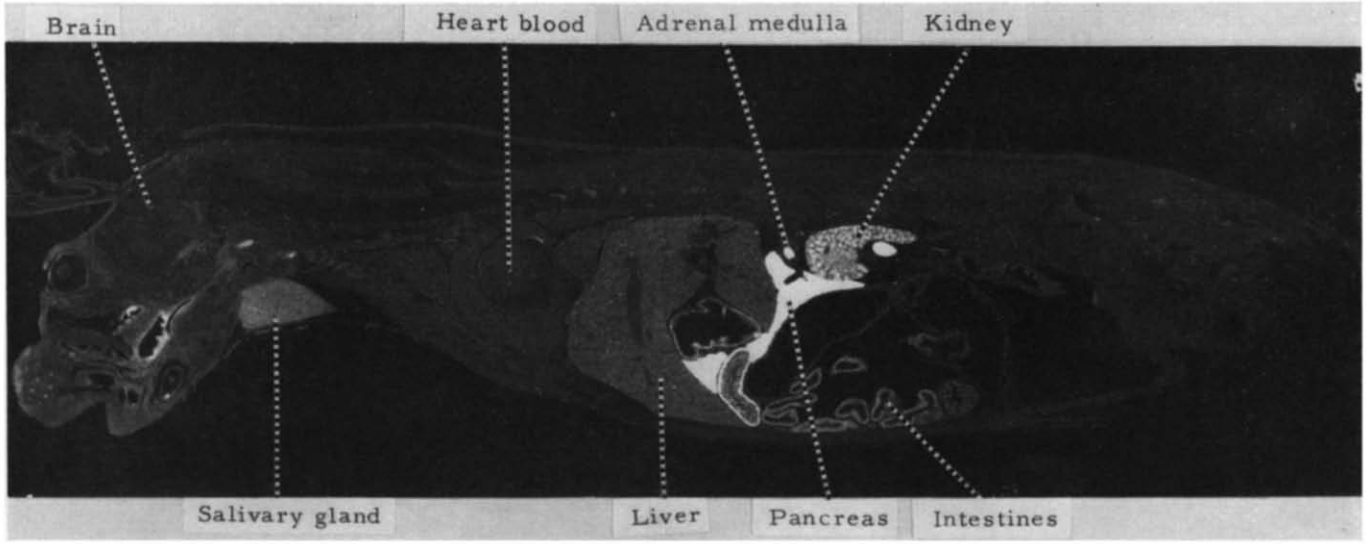

FIG. 1. Autoradiogram from mouse 40 min after intravenous injection of 2-^{14}C-dopa. Note high concentration in organs with rapid protein synthesis such as pancreas. Activity is also accumulating in the adrenal medulla.

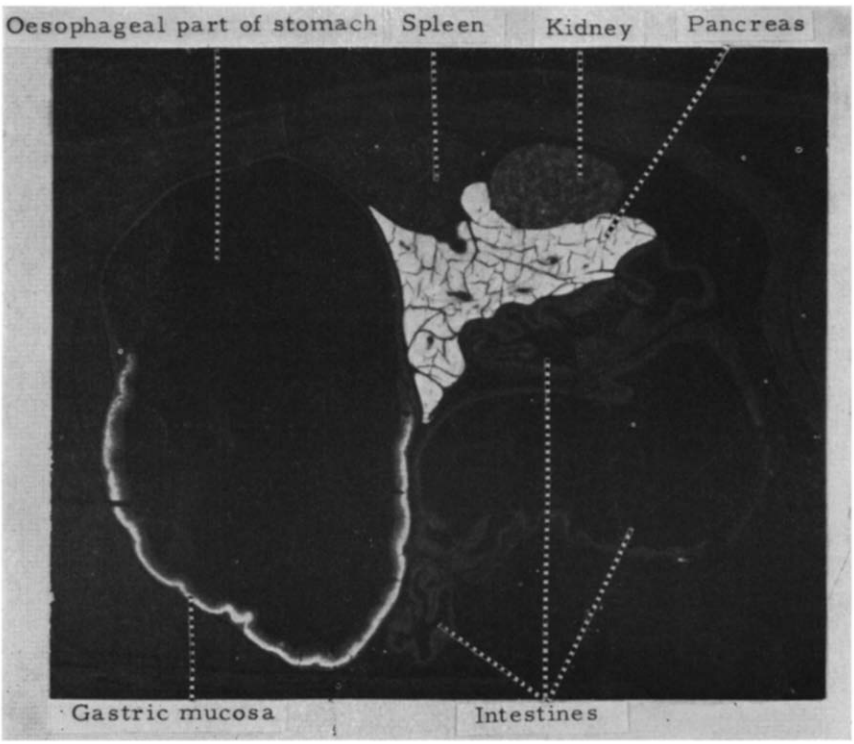

FIG. 2. Autoradiogram from abdominal region of mouse killed 1 hr after injection of 2-^{14}C-dopa
Note high activity in basal layer of gastric mucosa and in pancreas.

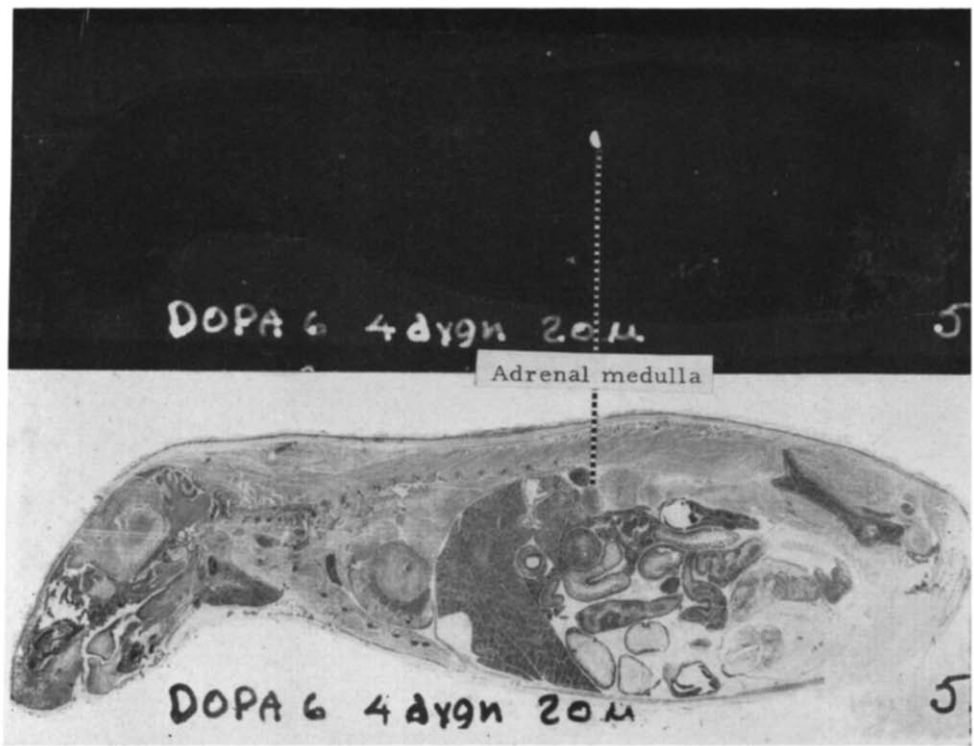

FIG. 3. *Above*, autoradiogram and, *below*, corresponding section of mouse killed 4 days after injection of ^{14}C-dopa. The radioactivity has disappeared from most organs but a strong and selective accumulation is seen in the adrenal medulla. According to the chromatograms (see Fig. 5) the radioactivity in the adrenal now represents adrenaline and noradrenaline.

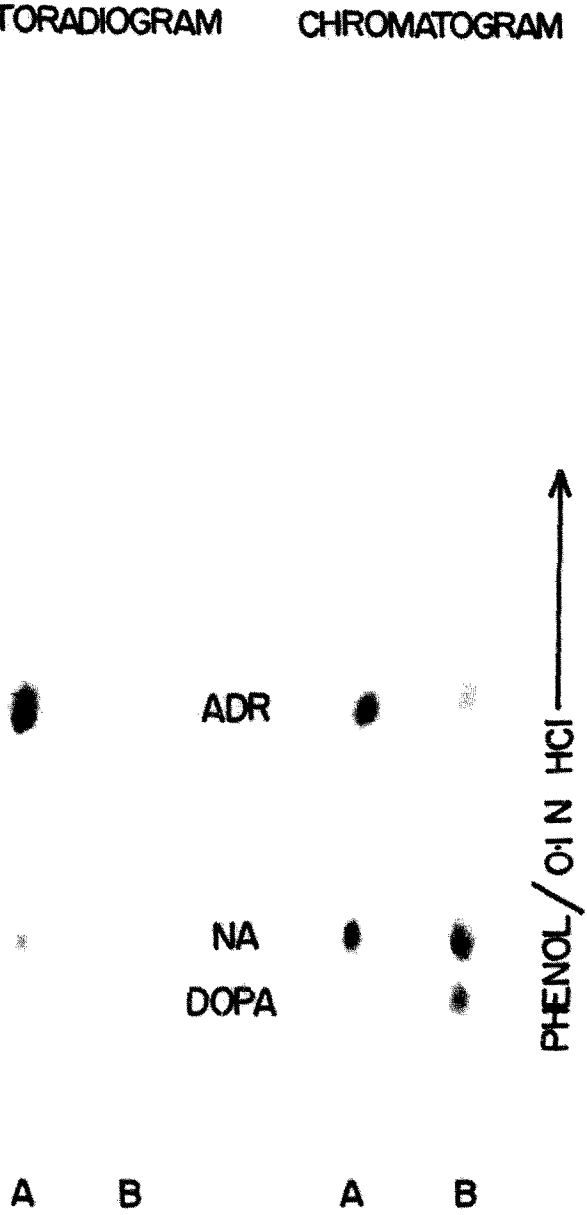

FIG. 4. Chromatographic separation of radioactive components of pancreas.
To the left is seen an autoradiogram of the stained paper chromatogram shown to the right.
A. Extract of pancreas 10 min after injection of ^{14}C-dopsa.
B. Extract of pancreas 30 min after injection of ^{14}C-dopa.
C. Reference substances (dopamine, dopa and noradrenaline) added to extract of mouse not injected with ^{14}C-dopa.
The radioactivity mainly represents dopa but also dopamine and other radioactive metabolites.

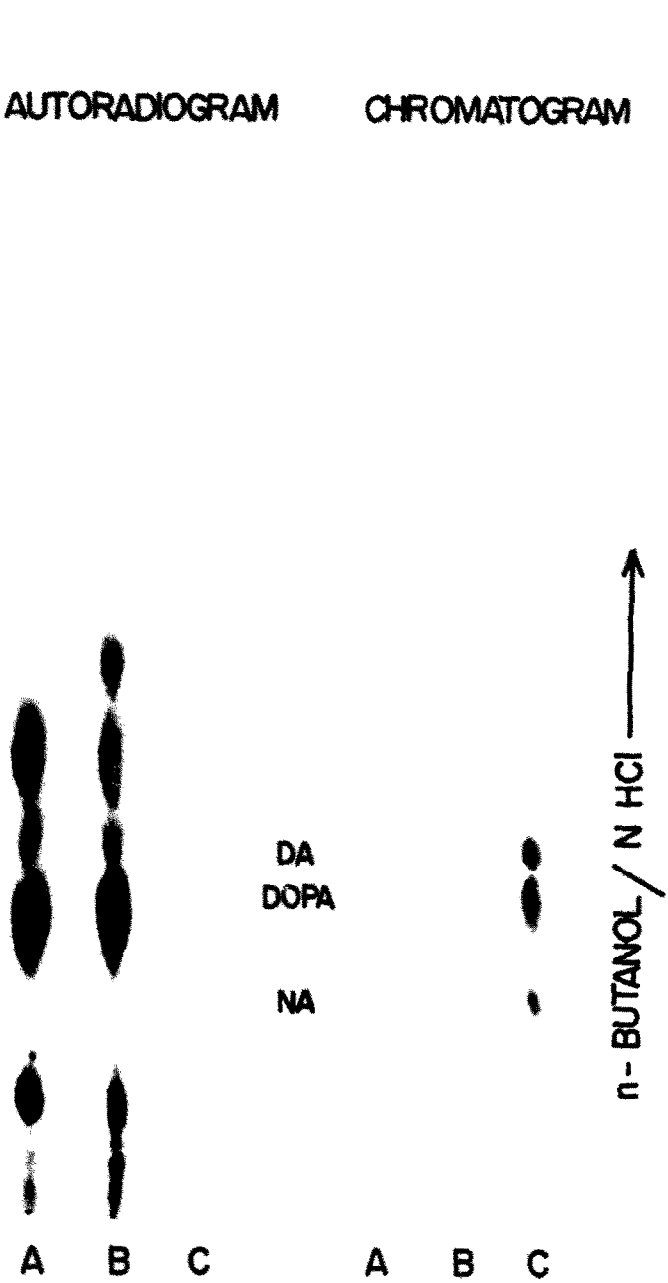

FIG. 5. Chromatographic separation of radioactive components of adrenal 4 days after injection of ^{14}C-2-dopa.

To the left is seen an autoradiogram of the chromatogram shown to the right. A = adrenal extract, B = reference substances (adrenaline, noradrenaline and dopa). The radioactivity of the adrenal now mainly represents adrenaline and to a lower extent noradrenaline.

RESULTS

A. Autoradiography

The ^{14}C-dopa was rapidly taken up from the blood by various tissues. Five minutes after injection, the highest concentration was found in the pancreas followed by the gastric mucosa, kidney, adrenal medulla, intestinal mucosa, salivary glands, bone marrow and liver. In the pancreas, the highest activity was found in the exocrine part and, in the gastric mucosa, the highest activity was found in the basal part of the glands. The radioactive substance penetrated freely into the brain.

After 20 min and 1 hr (Figs. 1 and 2), the activity in the adrenal medulla had increased tremendously and was now much higher than in pancreas. The pancreas and the other organs followed in about the same order as was found after 5 min. In the pregnant mouse, the foetuses showed a similar uptake to the mother 30 min after injection.

From this time onwards, the activity in the adrenal medulla continued to increase although at a slower rate and the radioactivity gradually disappeared from the other organs.

After 4 hr the adrenal medulla totally dominated the distribution picture. A fair amount of activity could also be seen in the excretory pathways, the urinary bladder and the biliary ducts and intestinal lumen, while the pancreas and other tissues only showed faint activity.

After 24 hr, slight activity still remained in the intestinal lumen and, after 4 days, radioactivity could be recognized only in the adrenal medulla (Fig. 3).

B. Chromatography

Pancreas. The identification of the radioactive components was based on the *Rf* values in the two chromatographic systems used.

At the two intervals studied, 10 and 30 min after injection of ^{14}C-dopa, the radioactive material on the chromatograms was separated in about seven relatively well-defined areas (Fig. 4). The dominating radioactive spot had the same *Rf*-value as dopa. One of the minor metabolites behaved as dopamine in both the systems used. None of the labelled components behaved as noradrenaline. The proportion between ^{14}C-dopa and ^{14}C-dopamine was not significantly changed from 10 to 30 min. No attempt was made to identify the other radioactive metabolites.

Adrenal medulla. Ten minutes after injection of ^{14}C-dopa, only two radioactive components were found on the chromatograms. These were identified as dopa and dopamine.

Thirty minutes after injection, ^{14}C-dopa had disappeared almost completely while ^{14}C-dopamine and ^{14}C-noradrenaline seemed to be present in about equal amounts. No signs of radioadrenaline in the adrenal gland were found at these early times studied.

Four days after the injection of ^{14}C-dopa, most of the radioactivity represented adrenaline but noradrenaline was also present (Fig. 5).

No radioactive spots besides the above-mentioned ones could be detected.

DISCUSSION

Our investigation has shown that, after injection of ^{14}C-2-dopa, radioactivity initially accumulates in organs characterized by a rapid protein synthesis and also in the adrenal

medulla. Later on, the radioactivity vanishes from most tissues but increases in the adrenal.

The selective accumulation in the adrenal medulla and, as shown by the chromatograms, transformation of ^{14}C-dopa to ^{14}C-adrenaline, add a good illustration to the generally accepted role of dopa as catecholamine precursor.

It is interesting to note that, 30 min after the injection of dopa, the adrenal medulla contained noradrenaline but not adrenaline. This finding indicates a slow synthesis of adrenaline from noradrenaline and confirms earlier studies which indicate a slow synthesis of adrenaline in relation to noradrenaline after the adrenal medulla has been depleted of catecholamines.[9]

The early distribution pattern of dopa is very similar to that found with the same autoradiographic technique using protein-forming amino acids.[10] A similar distribution picture has also been obtained with the amino-acid analogue parafluorophenylalanine.[11]

The explanation for the fact that dopa is distributed as a protein-forming amino acid is probably that the cell uptake of amino acids is not very selective but that the next step, the incorporation into proteins, involves a more accurate discrimination.

When labelled amino acids are incorporated into structural proteins, the radioactivity is retained with a long biological half-life. The rapid disappearance of ^{14}C-dopa from the tissues indicates that it is not incorporated into proteins. Dopa therefore seems to be a very suitable tool for studies on the mechanism of uptake into cells of amino acids without interference by the process of incorporation into proteins.

The rate of uptake and disappearance of ^{14}C-dopa was rather similar for all extra-adrenal tissues. The brain also lost its radioactivity at about the same rate as the other organs. A pertinent question which arises in connection with the disappearance of ^{14}C-dopa from the organs is whether the unchanged dopa to any large extent returns to the blood or whether it is decarboxylated or metabolized in some other way before it leaves the organs.

It is difficult to draw any conclusions in this matter from our chromatographic investigation in which pancreas has been chosen as representing the "protein synthesizing organs". The proportion between ^{14}C-dopa and ^{14}C-dopamine was not changed significantly from 10 to 30 min after injection. However, dopamine may return to the blood stream soon after decarboxylation.

Whether the bulk of noradrenaline and adrenaline found in the adrenal derives from dopa taken up directly by the organ or from dopamine obtained by decarboxylation is another difficult question. This investigation points at the possibility that the dopamine which has been formed by decarboxylation in the "protein synthesizing organs" may constitute a considerable part of the precursor substance which is collected at the sites of adrenaline and noradrenaline formation.

An investigation by Rosell and Sedvall[12] indicates that dopa is not, or only to a minor extent, decarboxylated by sympathetic nerves *in vivo*. It was found that noradrenaline and dopamine could restore the effect of vasoconstrictor nerve stimulation in reserpinized cats after intra-arterial administration. A weak restoring effect was found also for dopa but the threshold dose was about 100 times larger than for the catecholamines.

Experiments by Pennefather and Rand[13] have indicated that part of the decarboxylation of injected dopa is located to some gastrointestinal structure. They found that

evisceration prevented the rise in noradrenaline equivalent in kidney, uterus and spleen which was obtained in intact animals after infusion with l-dopa.

The amount of ^{14}C-dopa which in our chromatographic investigation was found in the adrenal 10 min after injection might not have been larger than the amount taken up by many other tissues.

The time lag in uptake by the adrenal medulla in relation to the pancreas may suggest that the quantitatively dominating precursor taken up by the adrenal medulla is dopamine and not dopa. However, this question demands further investigation.

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