Br. J. Pharmac. (1970), **40**, 461–467.

Distribution and metabolism of L-3-O-methyldopa in rats

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Summary

1. After intraperitoneal administration of L-2-^{14}C-3-O-methyldopa (^{14}C-O-methyldopa) to rats, the amino-acid was distributed evenly in blood, brain, heart, adipose tissue and liver, whereas it accumulated more in the kidney and the pancreas. ^{14}C-O-methyldopa showed a biological half-life of about 12–13 h in blood, brain and heart.

2. The concentration curve of ^{14}C-O-methyldopa in brain (after increasing doses of the amino-acid) was linear if measured 2 h after administration, but seemed to reach a plateau at the higher doses if determined after 16 h.

3. The concentrations of ^{14}C-O-methyldopa metabolites (mainly homovanillic acid and 4-hydroxy-3-methoxyphenyllactic acid) were low, except in the kidney, and varied according to the tissue.

4. Twenty-four hours after administration of ^{14}C-O-methyldopa, 33% of the injected radioactivity appeared in the urine. This radioactivity consisted of about 95% of metabolites (probably in the main ^{14}C-homovanillic acid and ^{14}C-4-hydroxy-3-methoxyphenyllactic acid) and of 5% of unchanged ^{14}C-O-methyldopa. In the faeces, 10% of the radioactivity appeared, mainly as metabolic end-products.

5. It is concluded that ^{14}C-O-methyldopa easily penetrates from the blood into various tissues, including brain, and that the majority of the amino-acid undergoes a slow metabolism. The different shape of the concentration curves for ^{14}C-O-methyldopa in the brain after 2 and 16 h might indicate the presence of two tissue pools of the amino-acid.

Introduction

After a single dose or repeated administration of L-dopa to animals, a considerable accumulation of 3-O-methyldopa (4-hydroxy-3-methoxyphenylalanine) occurs in the brain and heart (Bartholini & Pletscher, 1968 ; Kuruma, Bartholini & Pletscher, 1970). The disappearance of 3-O-methyldopa from these organs is rather slow, as the compound can still be detected 48 h after a single injection of L-dopa. In contrast, the other L-dopa metabolites, for example catecholamines, phenolcarboxylic acids and dopa itself, disappear much faster, especially from the brain (Kuruma *et al.*, 1970). Because 3-O-methyldopa has also been discovered in the blood and cerebrospinal fluid of humans treated with L-dopa (Pletscher, Bartholini & Tissot, 1967 ; Tissot, Bartholini & Pletscher, 1969) and because high doses of L-dopa are used in the treatment of Parkinson's syndrome, a closer investigation of the fate of

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3-O-methyldopa in the organism seems to be indicated. It has, for instance, not yet been decided whether this metabolite is formed within the brain and the heart from L-dopa or whether it penetrates mainly from the blood into these organs. Furthermore, the reason for the slow disappearance of 3-O-methyldopa from the tissues is not clear.

In order to elucidate these problems, experiments on the tissue distribution and metabolism of radioactive L-3-O-methyldopa have been carried out in rats.

Methods

Male albino rats (80–100 g) of Wistar origin (Füllinsdorf) were injected intraperitoneally with 1 mg/kg L-2-^{14}C-3-O-methyldopa (^{14}C-O-methyldopa) (specific activity 193 μCi/mg) and killed at different time intervals after administration of the amino-acid (time curve). In another series, varying amounts of ^{14}C-O-methyldopa (specific activity 6·2–193 μCi/mg) were similarly injected 2 or 16 h before killing. The organs, the faeces excreted within 24 h, and the plasma obtained from heparinized blood were homogenized with 0·4 M perchloric acid, centrifuged, and the radioactive metabolites in the supernatant fluid were fractionated on Dowex 50-X4 as previously described (Bartholini & Pletscher, 1968). An aliquot of the pooled 24 h urine collected in metabolic cages from six rats was treated in the same way as the supernatant fluids. Two radioactive fractions were obtained: (*a*) the effluent containing mainly metabolic end-products such as neutral, acidic and conjugated derivatives, and (*b*) the fraction containing amino-acids which were absorbed on the column and re-eluted with 0·1 M potassium acetate buffer, pH 6·0. In a third fraction, eluted with 2 N HCl, which normally contains the catecholamines and their O-methylated derivatives, only small amounts of radioactivity could be found, which were not considered.

An aliquot of the fraction containing the metabolic end-products, adjusted to pH 5 with KOH, was incubated with glusulase (containing 50,000 u. β-glucuronidase and 400,000 u. sulphatase) for 2 h at 37° C under nitrogen. The reaction was stopped by addition of HCl until pH 1 was reached, and 20 min later the mixture was readjusted to pH 5 and centrifuged in order to remove the proteins. The supernatant fluid was passed through a column of Dowex AG3-X4 (0·5 g), and the phenylcarboxylic acids were eluted with 20 ml 5 N acetic acid. Aliquots of this eluate and of the amino-acid fraction eluted from the Dowex 50-X4 were measured for radioactivity in a liquid scintillation counter. The remaining portion of the eluates was evaporated and the residues dissolved in 75% (v/v) aqueous methanol for paper chromatography on Whatman No. 1 using butan-1-ol saturated with 0·1 M potassium acetate buffer pH 4·5 and on Schleicher & Schuell No. 2043 paper using ethyl acetate : acetic acid : water (5 : 1·5 : 3 by volume). The paper chromatograms were cut into strips, and the radioactivity of each strip was measured in a liquid scintillation counter.

Results

Effect of dose

Intraperitoneal injection of ^{14}C-O-methyldopa induced a dose-dependent rise in the radioactivity of the fraction containing ^{14}C-amino-acids from the brain of rats. The concentration of ^{14}C-O-methyldopa measured in the brain 2 h after administration showed a linear increase with dose. Determination after 16 h showed that the

absolute concentrations of ^{14}C-O-methyldopa were considerably lower at all doses and tended to reach a plateau (Fig. 1). Paper chromatography indicated that over 95% of the fraction containing amino-acids consisted of ^{14}C-O-methyldopa.

Time course

A maximal accumulation of ^{14}C-O-methyldopa in brain, heart and blood plasma was observed between 0·5 and 2 h after intraperitoneal administration of 1 mg/kg of the amino-acid. Thereafter, the ^{14}C-O-methyldopa slowly declined, but remained detectable for at least 48 h (Fig. 2). Maximal concentrations and time courses were similar in brain, heart and blood plasma. The biological half-life of ^{14}C-O-methyldopa was about 12–13 h in all three tissues.

Organ distribution

Four hours after intraperitoneal administration of 1 mg/kg ^{14}C-O-methyldopa, about equal concentrations of the amino-acid per gram tissue (2·2–2·5 nmol/g) were found in blood plasma, liver, heart, brain and adipose tissue. Kidney and pancreas, however, showed considerably higher values, 6·5 and 20·3 nmol/g respectively (Table 1).

Metabolism

The concentration of the metabolic end-products 4 h after injection of 1 mg/kg ^{14}C-O-methyldopa varied in the different tissues. The highest accumulation occurred

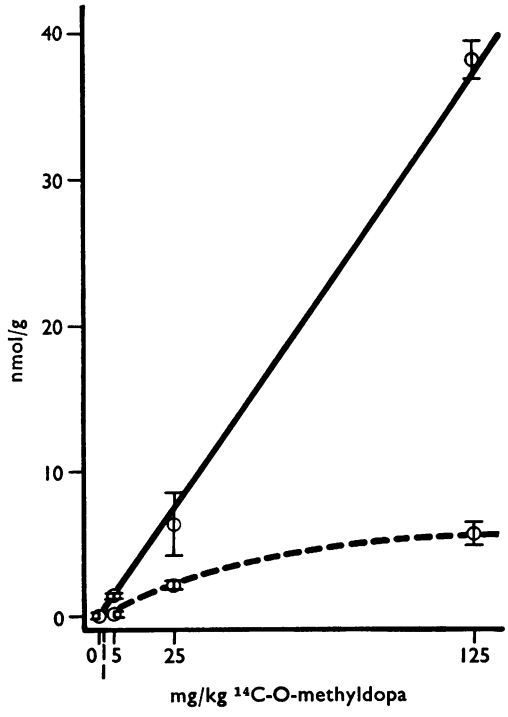

FIG. 1. Concentration of ^{14}C-O-methyldopa in rat brain 2 h (——) and 16 h (– – –) after intraperitoneal administration of various doses of ^{14}C-O-methyldopa. The points indicate mean results from three experiments with S.E.

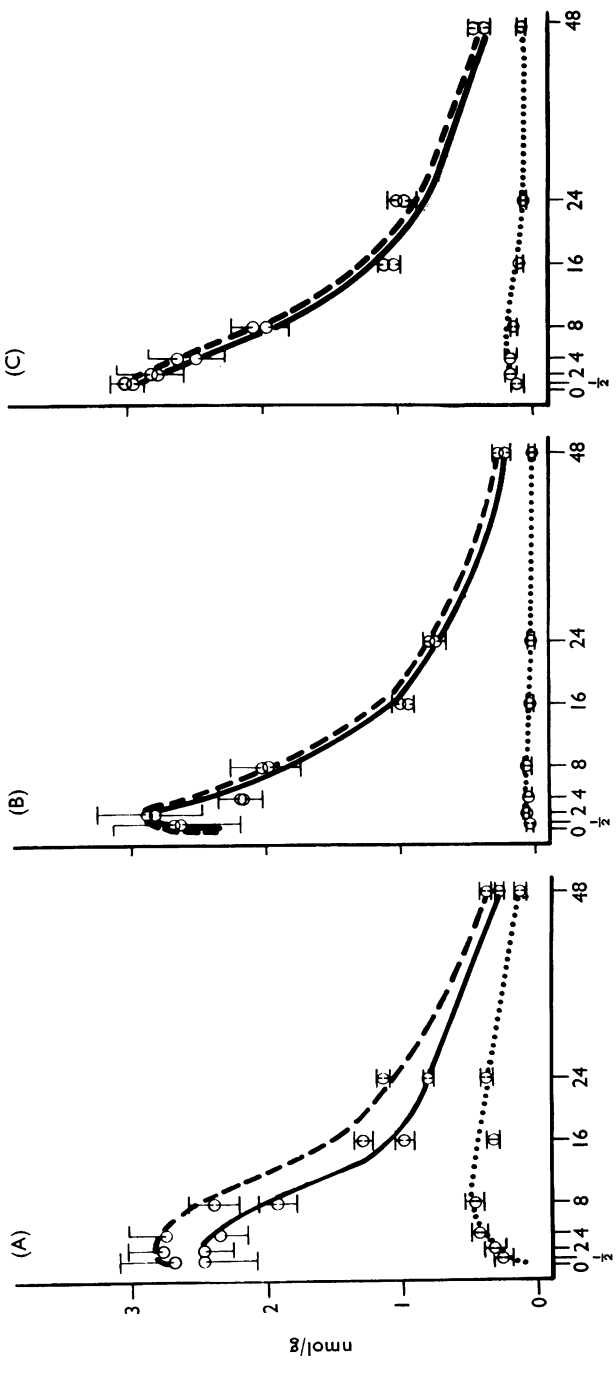

FIG. 2. Time course of ^{14}C-O-methyldopa and its metabolic end-products in blood (A), brain (B) and heart (C) of rats after intraperitoneal administration of 1 mg/kg ^{14}C-O-methyldopa. The points indicate mean results with S.E. from three experiments. ----, Overall radioactivity; ——, ^{14}C-amino-acids (mainly ^{14}C-O-methyldopa); ·····, ^{14}C-metabolic end-products.

in the kidney which contained 4·9 nmol/g, accounting for 45% of the radioactivity found in this organ. Considerably lower concentrations of 0·4–0·5 nmol/g, amounting to 16–17% of the total radioactivity, were present in the blood plasma and liver. The lowest concentration of metabolites was found in the brain, where the concentration of 0·05 nmol/g represented only 2% of the total radioactivity (Table 1). The maximum concentration of metabolic end-products in the blood plasma (0·5 nmol/g) was attained 4–8 h after administration of 1 mg/kg ^{14}C-O-methyldopa. Subsequently the concentration of the metabolites slowly declined, but was still measurable after 48 h (Fig. 2). In the brain and the heart the time course for the metabolites was similar to that in the blood, but their absolute concentration was lower at all time intervals measured; for example, it was 0·05 and 0·2 nmol/g respectively after 4 h (Fig. 2, Table 1). Paper chromatography indicated that the fraction containing phenolcarboxylic acids from the blood plasma, after enzymatic hydrolysis, consisted of two main components, 4-hydroxy-3-methoxyphenyllactic acid (74·2 ± 1·5%) and homovanillic acid (4-hydroxy-3-methoxyphenylacetic acid) (25·8 ± 1·5%). Similar results were obtained from heart and brain.

In the urine, 33 ± 0·3% of the injected radioactivity was detected after 24 h (two experiments with a pool of six rats each). Only 5 ± 0·2% of this urinary radioactivity consisted of ^{14}C-O-methyldopa, whereas the majority appeared in the fraction of metabolic end-products. Preliminary results indicate that this fraction contains mainly homovanillic acid and 4-hydroxy-3-methoxyphenyllactic acid. In the faeces, 9·3 ± 0·6% of the injected radioactivity is eliminated within the first 24 h (two experiments, each with pooled samples from four rats). More than 95% of this radioactivity is present as metabolic end-products.

Discussion

According to the time curves obtained after intraperitoneal injection of ^{14}C-O-methyldopa, this amino-acid is distributed rather uniformly in blood plasma, brain and heart. The distribution in liver and adipose tissue seems to be similar, whereas the amino-acid accumulated more in the pancreas and kidney. These findings indicate that methyldopa penetrates easily from the blood into various organs including brain. Therefore, the accumulation of ^{14}C-O-methyldopa (Bartholini & Pletscher, 1968; Kuruma *et al.*, 1970) in brain and heart observed after administration of ^{14}C-L-dopa may at least in part be due to penetration of ^{14}C-O-methyldopa from the blood into these organs rather than to intracerebral or intracardiac 3-O-methylation of ^{14}C-L-dopa. The ^{14}C-O-methyldopa found in the blood after ^{14}C-L-dopa

TABLE 1. *Distribution of total radioactive compounds (TRC), ^{14}C-amino acids (mainly ^{14}C-O-methyldopa) and ^{14}C-metabolic end-products in various organs of the rat 4 h after intraperitoneal administration of 1 mg/kg ^{14}C-O-methyldopa*

Tissue	Total radioactive compounds nmol/g	O-Methyldopa nmol/g	O-Methyldopa % of TRC	Metabolites nmol/g	Metabolites % of TRC
Liver	2·7±0·2	2·4±0·2	87±1	0·5±0·1	17±4
Kidney	10·8±0·1	6·5±0·1	60±2	4·9±0·5	45±4
Adipose tissue	2·6±0·4	2·4±0·4	95±1	0·2±0·0	6±1
Pancreas	20·0±1·7	20·3±1·9	101±1	0·4±0·1	2±0
Heart	2·6±0·2	2·5±0·2	95±0	0·2±0·0	6±0
Brain	2·2±0·2	2·2±0·1	99±1	0·05±0·0	2±0
Blood plasma	2·8±0·3	2·4±0·2	85±1	0·4±0·0	16±0

The values represent mean results with S.E. from three experiments each performed in duplicate.

administration probably has its principal origin in the liver, which is known to be rich in catechol-3-O-methyltransferase (Axelrod, Albers & Clemente, 1959). The reason for the greater accumulation of methyldopa in pancreas and kidney than in other organs remains to be investigated.

The present experiments with ^{14}C-O-methyldopa confirm previous findings with ^{14}C-L-dopa (Pletscher *et al.*, 1967 ; Bartholini & Pletscher, 1968 ; Tissot *et al.*, 1969 ; Kuruma *et al.*, 1970) which indicated that the methyldopa formed from L-dopa in the tissues disappears rather slowly. The biological half-life of ^{14}C-O-methyldopa in brain and heart is much longer than that of ^{14}C-L-dopa (12–13 h compared with about 0·5 h) (Bartholini & Pletscher, 1968). This relatively slow disappearance cannot be explained by a high affinity for lipids, for the ^{14}C-O-methyldopa does not preferentially accumulate in adipose tissue. The reasons for the relatively long biological half-life of ^{14}C-O-methyldopa may, however, lie in its poor elimination and its slow metabolism. In fact, only small amounts of unchanged ^{14}C-O-methyldopa appear in the urine and the faeces in spite of persisting elevated blood levels of the compound. The bulk of the radioactivity is excreted in the urine in the form of ^{14}C-O-methyldopa metabolites. These seem to be formed rather slowly. Thus, less than 50% of the injected radioactivity is excreted as metabolites within 24 h. Also, throughout the whole time course of the experiments (0–48 h), only relatively small amounts of metabolic end-products are found in the blood and even less in the brain and heart. Furthermore, in the blood the maximal concentrations of the metabolic end-products are reached only 4–8 h after administration of ^{14}C-O-methyldopa. In contrast, as shown previously (Bartholini & Pletscher, 1968), the degradation of ^{14}C-L-dopa seems to be much more rapid, since large amounts of ^{14}C-phenolcarboxylic acids appear in blood, brain and heart shortly after administration of the amino-acid.

The slow metabolism of ^{14}C-O-methyldopa is possibly connected with the finding that this compound, in contrast to L-dopa, represents a poor substrate for the decarboxylase of aromatic amino-acids (Ferrini & Glässer, 1964). It cannot be excluded, however, that part of the ^{14}C-homovanillic acid found in blood and urine has been formed by direct decarboxylation and subsequent deamination of ^{14}C-O-methyldopa, but the acid might also be a product of other metabolic pathways, for example transamination. Indeed, the occurrence of ^{14}C-4-hydroxy-3-methoxyphenyllactic acid as a major metabolite shows that transamination takes place to a considerable extent. It is of interest that the absolute concentration of ^{14}C-O-methyldopa metabolites and their proportion of the total radioactivity in the tissue shows large differences. Whether these are due to differences in the rates of metabolism of ^{14}C-O-methyldopa or to different elimination rates of the ^{14}C-metabolites remains to be investigated.

The dissimilar shapes of the concentration curves of ^{14}C-O-methyldopa measured in the brain 2 and 16 h after administration of the amino-acid (Fig. 1) may indicate its localization in at least two pools. The linear rise after 2 h possibly reflects an unspecific distribution of ^{14}C-O-methyldopa in the extra- and possibly the intracellular fluid, the concentration of which is in equilibrium with that of plasma and thus directly dependent on the administered dose of ^{14}C-O-methyldopa. The ^{14}C-O-methyldopa which remains after 16 h in the brain may occupy more specific sites that become saturated at the higher dose levels, explaining the tendency to form a plateau.

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(*Received April* 13, 1970)