J. Pharm. Pharmacol. 1982, 34: 719–723
Received April 5, 1982

0022–3573/82/110719–05 $02.50/0

Transamination and other metabolic pathways of 3,4-dihydroxyphenylpyruvic acid in rats when simultaneously administered with L-dopa

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In the rat, 3,4-dihydroxyphenylpyruvic acid (DHPPA) was shown to be transaminated to L-dopa when administered orally concomitantly with L-[^{3}H]dopa. As much as 40% of the serum L-dopa and brain dopamine was shown to be formed via transamination. Moreover, it was shown that DHPPA also caused a so-called L-dopa-sparing effect, i.e. more administered L-dopa reached the circulation and consequently more dopamine reached its target than after administration of L-dopa alone. This effect might be due to an inhibition of the deamination of L-[^{3}H]dopa, since in this case the equilibrium of the transamination reaction will be forced into the L-dopa-forming direction. After administration of [^{14}C]DHPPA alone and together with L-dopa, no differences in the absorption and distribution of radioactivity were found.

In previous studies (Hietala et al 1979; Lindén 1980) we have shown that the concomitant administration of L-dopa and its corresponding α-keto acid, 3,4-dihydroxyphenylpyruvic acid (DHPPA), to rats significantly increased the bioavailability of L-dopa. Likewise, we could show that glutamic acid, which has been reported to act as a donor of amino groups in the transamination of DHPPA (Maeda & Shindo 1977), also had an L-dopa-sparing effect when administered together with L-dopa. Therefore, the L-dopa-sparing effect obtained by coadministration of DHPPA or glutamic acid was proposed, at least partly, to be due to the equilibrium of the L-dopa transamination reaction being forced into the L-dopa forming direction.

In the present study, experiments with L-[^{3}H]dopa and [^{14}C]DHPPA were designed to elucidate to what extent DHPPA is transaminated to L-dopa in the rat when administered simultaneously. The absorption and distribution as well as the metabolic fate of DHPPA in the brain were compared with those of L-dopa.

MATERIALS AND METHODS

Radioactive compounds

3,4-Dihydroxyphenyl[2-^{14}C]pyruvic acid ([^{14}C]-DHPPA). Labelled DHPPA was prepared starting from [2-^{14}C]glycine, 0·5–2·0 mCi (The Radiochemical Centre, Amersham) in five separate syntheses by modifying the methods of Maeda & Shindo (1977) and Harington & Randall (1931). After acetylation and reaction with protocatechualdehyde the intermediate 3-diacetoxyphenyl-2-acetylamino[2-^{14}C]acrylic acid ([^{14}C]DAAA) was obtained and thoroughly purified by extraction with ethyl acetate, re-extraction from chloroform with water and again with ethyl acetate. Final purity was achieved by preparative chromatography on thick layer plates in the solvent system chloroform–acetone–methanol–acetic acid–water (50:20:10:10:5). The radiochemical purity of [^{14}C]DAAA was over 99%.

Hydrolysis of [^{14}C]DAAA was effected by gentle boiling in a mixture of H_2SO_3 (5–6%) and HCl (concn.) (19:1, v/v) for 4 h. [^{14}C]DHPPA extracted with ethyl acetate was obtained as an off white powder; the radiochemical purity varied between 85 and 95% and spec. act. between 0·2–0·7 mCi mmol^{-1}.

L-3,4-Dihydroxyphenyl[2,3-^{3}H]alanine (L-[^{3}H]-dopa) was purchased from The Radiochemical Centre, Amersham.

3,4-Dihydroxyphenylpyruvic acid (DHPPA) was synthesized according to the method of Harington & Randall (1931) in the Research Laboratories of Medica Ltd, Helsinki, Finland.

L-3,4-Dihydroxyphenylalanine (L-dopa) was a commercial product of Medica Pharmaceutical Company Ltd, Helsinki, Finland.

All reagents were of p.a. quality.

Determination of L-dopa and dopamine

Animal experiments. Male Sprague-Dawley rats, 190–250 g, fasted for 18 h, were given oral doses

* Correspondence.

(mg kg^{-1}) of the following compounds: L-[^{3}H]dopa (100 mg, 100 μCi); L[^{3}H]dopa (100 mg, 100 μCi) + DHPPA (300 mg); [^{14}C]DHPPA (300 mg, 28–59 μCi); [^{14}C]DHPPA (300 mg, 26–69 μCi) + L-dopa (100 mg).

The ratio 1:3 for L-dopa + DHPPA was chosen because it was found in a previous study to have the best L-dopa-sparing effect. The drugs were administered as 5% gum arabic suspensions. Blood samples were taken from the tail artery 1 h after the oral doses. Thereafter the rat was decapitated, the brain removed, immediately divided into two equal parts and weighed. The brain and serum samples were kept at −20 °C until analysed fluorometrically and by radiochromatography for L-dopa, dopamine and some metabolites of L-dopa. Other organs were excised, weighed and homogenized. The samples were counted for radioactivity in a liquid scintillation counter (Wallac Rack Beta LSC 1215). Student's *t*-test was used for statistical analyses of the results.

Fluorometric determination of L-*dopa and dopamine.* The serum and brain samples were extracted and assayed according to the method described by Hietala et al (1979).

Determination of labelled dopamine in rat brain. Extraction of dopamine: The method described by Hietala et al (1979) was used. The only modification was the use of 0·5 ml H_2SO_3 (5–6% SO_2) instead of phosphate buffer (0·5 M, pH 6·5) for the elution of dopamine from Al_2O_3.

Thin-layer radiochromatography: 250 μl of the eluted sample was separated by thin-layer chromatography (t.l.c.). The solvent system used was: n-butanol–water–acetic acid, 63:27:10. Polygram cel 300 UV_{254} t.l.c. plates were used. After development, the plate was cut into 1 cm pieces and their radioactivity was determined in a liquid scintillation counter. Labelled and unlabelled dopamine standards were applied concurrently with the sample. The unlabelled dopamine spots were identified by visualization.

Separation and analysis of L-*dopa metabolites in brain.* The organic phase derived from the extraction of dopamine was used for the counting of its labelled acidic metabolites. Labelled dopamine was determined from Al_2O_3 eluate and labelled 3-*O*-methyldopa (3-OMD) from the Al_2O_3 effluent.

Determination of labelled L-*dopa in rat serum.* Serum proteins were precipitated with 0·4 M $HClO_4$. After centrifugation at 10 000 *g* for 10 min, 5 ml of the supernatant was added to a tube containing 0·5 g Al_2O_3 and 0·25 g EDTA. The pH of the mixture was adjusted to 8·5–8·8. The sample was centrifuged and the supernatant was removed, whereafter the Al_2O_3 was washed with 5 ml H_2O. L-Dopa was eluted from Al_2O_3 with 2·0 ml H_2SO_3 (5–6% SO_2). 100 μl of the L-dopa containing sample was applicated on a polygram cel 300 UV_{254} t.l.c. plate. The solvent system used was: isopropanol–2M HCl, 65:35. The same procedure as for dopamine was used for identification and counting of L-dopa.

Disappearance of labelled L-*dopa and DHPPA from rat intestinal loop*

18 h fasted male Sprague-Dawley rats, 180–210 g, were anaesthetized with urethane. A 6 cm long intestinal loop, initiating 1 cm from the stomach, was ligated. The following compounds were injected into the loop in a volume of 0·5 ml: L-[^{3}H]dopa (1 mg, 2 μCi); L-[^{3}H]dopa (1 mg, 2 μCi) + DHPPA (1 mg); [^{14}C]DHPPA (1 mg, 0·5 μCi); [^{14}C]DHPPA (1 mg, 0·5 μCi) + L-dopa (1 mg). The loop was removed, emptied and washed with H_2O 5, 10, 20, 30 and 60 min after the injections. The radioactivity of the loop content and the homogenized intestinal wall was determined.

RESULTS

Fig. 1 A and 1 B show serum L-dopa and brain dopamine concentrations, determined in parallel by fluorometric and thin-layer radiochromatographic methods, 1 h after oral doses of L-[^{3}H]dopa and L-[^{3}H]dopa + DHPPA to rats. After an oral dose of 100 mg of L-[^{3}H]dopa alone the serum L-dopa and brain dopamine concentrations were equal as determined by both methods, whereas the combination, L-[^{3}H]dopa + DHPPA, gave fluorometric L-dopa and dopamine values significantly ($P<0·01$) higher

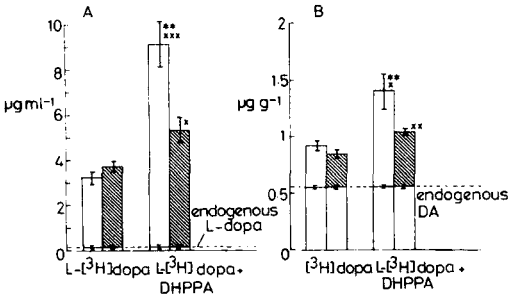

FIG. 1. A. L-Dopa concentrations in serum and B. Dopamine (DA) concentrations in brain of rats 1 h after oral doses of L-[^{3}H]dopa and L-[^{3}H]dopa + DHPPA. Open bars: fluorometric amounts. Hatched bars: labelled amounts. Each value is the mean ± s.e.m. of 6 determinations. Fluorometric versus radioactive value in the same treatment group $^{**}P<0·01$ L-[^{3}H]dopa + DHPPA versus L-[^{3}H]dopa treatment $^{***}P<0·001$, $^{**}P$ 0·01, $^{*}P<0·05$.

than the corresponding labelled values. This implies that a part of the L-dopa and dopamine has been formed via transamination of the unlabelled DHPPA. Furthermore, the labelled L-dopa and dopamine concentrations were significantly higher after L-[^{3}H]dopa + DHPPA than after L-[^{3}H]dopa alone.

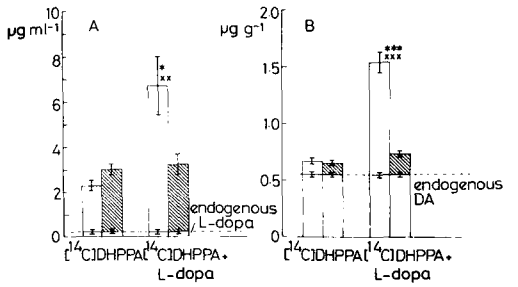

FIG. 2. A. L-dopa concentrations in serum and B, DA concentrations in brain of rats 1 h after oral doses of [^{14}C]DHPPA and [^{14}C]DHPPA + L-dopa. Open bars: fluorometric amounts. Hatched bars: labelled amounts. Each value is the mean ± s.e.m. of 4 determinations. Fluorometric versus radioactive values in the same treatment group $^{***}P < 0.001$, $^{*}P < 0.05$ [^{14}C]DHPPA + L-dopa versus [^{14}C]DHPPA treatment $^{***}P < 0.001$, $^{**}P < 0.01$.

Fig. 2 A and 2 B show serum L-dopa and brain dopamine concentrations, determined fluorometrically and by radiochromatography, 1 h after oral doses of [^{14}C]DHPPA and [^{14}C]DHPPA + L-dopa to rats. Part of the radioactivity administered as [^{14}C]DHPPA was found as L-[^{14}C]dopa in the serum, but only negligible amounts of it penetrated the blood-brain barrier. As about 40% of the L-dopa found in the serum after administration of [^{14}C]DHPPA + L-dopa is radioactive, [^{14}C]DHPPA is apparently transaminated to a considerable degree. This confirms the results with L-[^{3}H]dopa + DHPPA described above.

Table 1 shows that 44% more labelled L-dopa and 17% more total radioactivity was found in the serum after an oral dose of L-[^{3}H]dopa + DHPPA than after L-[^{3}H]dopa alone. The corresponding values for dopamine in the brain were 64 and 62% respectively. Likewise, 50% more labelled dopamine and 42% more total activity was found in the brain after [^{14}C]DHPPA + L-dopa than after [^{14}C]DHPPA alone, but no differences were found between the two treatment groups with regard to the serum L-dopa concentration and the total radioactivity.

Table 1. the difference in d min^{-1} expressed in per cent for total radioactivity, L-dopa and dopamine (DA) when L-[^{3}H]dopa + DHPPA treated rats are compared with L-[^{3}H]dopa treated and [^{14}C]DHPPA + L-dopa treated rats are compared with [^{14}C]DHPPA treated.

	Serum total activity	L-Dopa	Brain total activity	DA
L-[^{3}H]Dopa + DHPPA vs L-[^{3}H]dopa	+17	+44	+64	+62
[^{14}C]DHPPA + L-dopa vs [^{14}C]DHPPA	−10	+0·9	+42	+50

Table 2. Brain L-dopa metabolites, expressed as per cent of total radioactivity in brain 1 h after oral doses of L-[^{3}H]dopa, L-[^{3}H]dopa + DHPPA, [^{14}C]DHPPA and [^{14}C]DHPPA + L-dopa. Each value is the mean ± s.e.m. of 3–6 determinations.

Compound	% Acidic DA metabolites	DA	3-OMD
L-[^{3}H]dopa	44·6 ± 2·4	14·0 ± 0·7	41·2 ± 3·0
L-[^{3}H]dopa + DHPPA	54·7 ± 0·9	17·9 ± 1·9	27·4 ± 2·5
[^{14}C]DHPPA	23·6 ± 1·9	11·2 ± 0·8	65·2 ± 1·8
[^{14}C]DHPPA + L-dopa	41·1 ± 4·7	15·1 ± 1·5	43·7 ± 3·2

Table 2 shows the per cent distribution of the radioactive L-dopa metabolites: dopamine, 3-OMD and the acidic dopamine metabolites (mainly homovanillic acid and dihydroxyphenylacetic acid) after the four drug treatments mentioned. After administration of [^{14}C]DHPPA, as much as 65% of the radioactivity was found in the fraction containing 3-OMD. Correspondingly, the concentrations of dopamine and its acidic metabolites were low. On the other hand, after a dose of L-[^{3}H]dopa + DHPPA, high concentrations of dopamine and its acidic metabolites were found, which indicates a good access of L-dopa to the brain.

The distribution of radioactivity in some organs 1 h after oral doses of L-[^{3}H]dopa, L-[^{3}H]dopa + DHPPA, [^{14}C]DHPPA and [^{14}C]DHPPA + L-dopa is shown in Table 3. No differences were found between the amounts of radioactivity in the intestinal tract following [^{14}C]DHPPA and [^{14}C]DHPPA + L-dopa. The radioactivity in the intestinal tract was 83 and 77% of the dose, respectively. After L-[^{3}H]dopa and L-[^{3}H]dopa + DHPPA the corresponding values were 55 and 54%. The low absorption of [^{14}C]DHPPA was reflected in the amount of radioactivity found in the organs. After a dose of L-[^{3}H]dopa alone, about 3–6 times more radioactivity was found in the organs and blood than after [^{14}C]DHPPA or [^{14}C]DHPPA + L-dopa.

Table 3. The distribution of radioactivity, expressed as per cent of total doses, in some organs of the rat after oral doses of [^{14}C]DHPPA, [^{14}C]DHPPA + L-dopa, L-[^{3}H]dopa, L-[^{3}H]dopa + DHPPA. The mean ± s.e.m. is given when at least 3 determinations were performed.

Tissue	% [^{14}C]DHPPA	% [^{14}C]DHPPA + L-dopa	% L-[^{3}H]dopa	% L-[^{3}H]dopa + DHPPA
Blood	0·29 ± 0·4	0·24 ± 0·08	1·66 ± 0·11	1·78 ± 0·34
Brain	0·006 ± 0·0008	0·008 ± 0·003	0·04 ± 0·005	0·07 ± 0·007
Adrenals	0·001 ± 0·005	0·005 ± 0·0002	0·01 ± 0·005	0·01
Heart	0·009 ± 0·0005	0·007 ± 0·001	0·05 ± 0·01	0·02
Liver	0·67 ± 0·12	0·63 ± 0·34	5·67 ± 0·42	3·03 ± 0·29
Lung	0·02 ± 0·0003	0·04 ± 0·01	0·25 ± 0·13	0·07
Intestinal tract	83·58 ± 3·48	77·11 ± 1·80	55·55 ± 1·26	54·12 ± 1·35
Kidney	0·31 ± 0·04	0·22 ± 0·06	0·80 ± 0·09	1·13
Pancreas	0·08 ± 0·01	0·08 ± 0·01	0·34 ± 0·06	0·19
Spleen	0·01 ± 0	0·01 ± 0·01	0·09 ± 0·01	0·02
Urine	0·24 ± 0·17	0·47 ± 0·34	—	2·17

Only a slight disappearance of [^{14}C]DHPPA from ligated rat intestinal loop in situ was demonstrated. Fig. 3 shows that about 80% of the injected radioactivity was still in the intestinal content 60 min after the injection of [^{14}C]DHPPA or [^{14}C]DHPPA + L-dopa, while only 20% remained in the loop 60 min after L-[^{3}H]dopa or L-[^{3}H]dopa + DHPPA. In the intestinal wall the values for [^{14}C]DHPPA were about 0·4% and for L-[^{3}H]dopa about 4·5%.

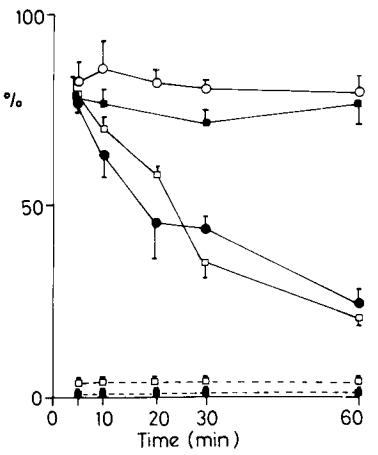

FIG. 3. Disappearance of radioactivity from rat intestinal loop (□ L-[^{3}H]dopa, ● L-[^{3}H]dopa + DHPPA, ■ [^{14}C]DHPPA, ○ [^{14}C]DHPPA + L-dopa) and intestinal wall (- - □ - - - [^{3}H]dopa - - ■ - - [^{14}C]DHPPA). Each value is the mean ± s.e.m. of 3 determinations.

DISCUSSION

We have earlier demonstrated an L-dopa-sparing effect of DHPPA in rats and mice after simultaneous administration of the two drugs. In this study, where labelled compounds were used, we showed that about 40% of the serum L-dopa and brain dopamine determined 1 h after an oral dose of L-[^{3}H]dopa + DHPPA originate from DHPPA. Likewise, we could demonstrate that when L-[^{3}H]dopa and DHPPA were given simultaneously, DHPPA not only was converted into L-dopa but also increased the bioavailability of L-[^{3}H]dopa. Significantly more labelled L-dopa and labelled dopamine were found after the combination than after L-[^{3}H]dopa alone.

Concerning transamination, the results obtained with labelled L-dopa + DHPPA were confirmed by the use of labelled DHPPA: [^{14}C]DHPPA was converted into L-[^{14}C]dopa. No significant differences were found between the serum concentrations of L-[^{14}C]dopa after doses of [^{14}C]DHPPA and [^{14}C]DHPPA + L-dopa.

Since DHPPA has been reported to be converted into L-dopa in intestinal homogenate without further metabolism to dopamine (Maeda & Shindo 1977) and the absorption of intact DHPPA is also reported to be poor (Maeda et al 1977), it seems evident that the gut is the main transamination site after oral doses of DHPPA and DHPPA + L-dopa, respectively. Accordingly the L-dopa-sparing effect demonstrated above must also occur mainly in the gut, where the greatest losses of L-dopa after oral doses have been reported to occur (Rivera-Calimlim et al 1970; Cotler et al 1976; Sasahara et al 1981). In serum the concentration of labelled L-dopa was 44% higher but the total radioactivity only 17% higher after L-[^{3}H]dopa + DHPPA than after L-[^{3}H]dopa alone. The explanation may be that, after all, some DHPPA reaches the circulation and to some extent prevents the peripheral L-dopa metabolism. The fact that the labelled dopamine concentration and the total radioactivity in the brain were about 60% higher after the combination than after L-[^{3}H]dopa alone supports this assumption.

Concomitant administration of DHPPA and L-dopa forces the transamination reaction into the L-dopa-forming direction which prevents deamination of L-dopa. The significant L-dopa-sparing effect mentioned earlier can either be due to the inhibition of the deamination route of L-dopa or to the weak dopa decarboxylase inhibition described for DHPPA (Gey & Messiha 1964). Besides, Tate et al (1971) have reported that L-dopa itself has a peripheral dopa decarboxylase inhibiting effect, after prolonged administration to mice and to patients with Parkinson's disease.

Although the labelled amount of L-dopa in serum was shown to be almost as high after 300 mg kg^{-1} of [^{14}]DHPPA as after 100 mg kg^{-1} of L-[^{3}H]dopa, only negligible amounts of labelled dopamine were found in brain after [^{14}C]DHPPA. This is in agreement with our results obtained in studies with unlabelled DHPPA. In a previous paper we demonstrated high concentrations of 3-OMD in rat serum after oral doses of DHPPA. As 3-OMD has been shown to have greater affinity for transport carriers in brain than L-dopa (Wade & Katzman 1976), we concluded that the poor penetration of L-dopa into the brain may be due to competition for the transport carriers. The present study supports this assumption, since we found significantly more radioactivity (65% of total in brain) in the 3-OMD fraction after [^{14}C]DHPPA alone than after the other three treatments. High concentrations of 3-OMD after intravenously administered [^{14}C]DHPPA have also been reported by Maeda et al (1977).

The distribution of radioactivity seems to follow the same pattern after all drug treatments studied. The degree of absorption, however, is lower for [^{14}C]DHPPA than for L-[^{3}H]dopa. The low absorption could possibly be due to the fact that intact DHPPA itself is poorly absorbed, which was confirmed with experiments on rat intestinal loops. Therefore, it is probably mostly that part of the administered DHPPA which is metabolized via L-dopa that reaches the circulation.

In conclusion, we have shown that [^{14}C]DHPPA administered concomitantly with L-dopa to rats is partly transaminated to L-dopa. With the transamination reaction forced into the L-dopa-forming direction, the deamination of the administered L-dopa is prevented. This does not, however, exclude the possibility that the L-dopa-sparing effect of DHPPA is due partly to the inhibition of dopa decarboxylase.

Acknowledgement

The skilful technical assistance of Mrs Ulla Manninen, Miss Tina Heikkinen and Miss Marita Sjoblom is gratefully acknowledged.

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