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METABOLISM AND BLOOD–BRAIN CLEARANCE OF L-3,4-DIHYDROXY-[^{3}H]PHENYLALANINE ([^{3}H]DOPA) AND 6-[^{18}F]FLUORO-L-DOPA IN THE RAT

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Abstract—6-[^{18}F]fluoro-L-DOPA (FDOPA) has been used as a tracer for the cerebral activity of L-3,4-dihydroxyphenylalanine (DOPA)-decarboxylase in studies of positron emission tomography (PET). However, the substitution of fluorine on the aromatic ring may alter the disposition and metabolism of FDOPA from that of endogenous DOPA. In the present study, the kinetics of the peripheral metabolism and the facilitated unidirectional blood–brain clearance of [^{3}H]DOPA and FDOPA were compared in Wistar rats pretreated with carbidopa. In arterial plasma, FDOPA was *O*-methylated with an apparent rate constant (0.031 min^{-1}) 3-fold that of [^{3}H]DOPA in the same rats. The *O*-methylated metabolite of FDOPA (OMe-FDOPA) was eliminated from plasma at a rate constant (0.018 min^{-1}) 3-fold that of OMe-[^{3}H]DOPA. The mean unidirectional blood–brain clearance of FDOPA (4.5 mL · hg^{-1} · min^{-1}) in six brain regions was 60% higher than that of [^{3}H]DOPA.

Key words: DOPA; 6-fluoro-L-DOPA; positron emission tomography; kinetics; catechol-*O*-methyltransferase; blood–brain permeability

FDOPA‡, a substrate for DOPA-decarboxylase, has been used as a metabolic tracer of dopamine synthesis [1] in PET studies. Like DOPA and other endogenous catechols, FDOPA in the circulation of human subjects is metabolized rapidly by COMT, yielding OMe-FDOPA [2]. Inhibition of COMT in peripheral tissues with U-0521 increases the availability of FDOPA for decarboxylation in the brain of living rats [3].

After simultaneous injection of FDOPA and [^{3}H]DOPA, OMe-FDOPA concentrations in plasma of carbidopa-pretreated rats are higher than OMe-[^{3}H]DOPA concentrations, suggesting that FDOPA is a better substrate for COMT *in vivo* [4], although DOPA has a much higher affinity for the enzyme *in vitro* [5]. However, this difference could reflect relatively impaired elimination, rather than preferential *O*-methylation of the fluorinated tracer. Therefore, we measured the kinetics of [^{3}H]DOPA and FDOPA metabolism in plasma of carbidopa-pretreated rats. The effect of ring-fluorination on the blood–brain permeability of DOPA is unknown. Therefore, the unidirectional blood–brain clearances of [^{3}H]DOPA and FDOPA were compared.

MATERIALS AND METHODS

All experiments were conducted in accordance with the McGill University Animal Ethics Committee. Male Wistar rats (250–300 g, Charles River, Québec) were anaesthetized with halothane (2%, Ayerst) and received heparinized lines in the right femoral artery and vein. Rats were immobilized in plaster casts for 30 min following surgery. L-3,4-[*ring*-2,5,6-^{3}H]Dihydroxyphenylalanine ([^{3}H]DOPA, 50 Ci/mmol) was obtained from New England Nuclear. FDOPA (200 Ci/mol at end of synthesis) was prepared by a modification, as reported previously [6], of the method of Luxen and Barrio [7].

For permeability studies, rats (N = 9) received [^{3}H]DOPA (50 μCi) and FDOPA (200 μCi), injected together as an intravenous bolus. Arterial blood samples were collected at 20-sec intervals until the rats were decapitated (1–3 min). Brains were dissected rapidly on ice into six regions (striatum, olfactory tubercle, parietal cortex, hippocampus, hypothalamus, and cerebellum), weighed, sonicated in 10 vol. of perchloric acid (0.1 M), placed on ice for 15 min, and centrifuged (14,000 *g* for 10 min). Plasma was separated from blood by rapid centrifugation, mixed with an equal volume of sulfo-salicylic acid [10% (w/v); Sigma], placed on ice for 10 min, and centrifuged. The gamma activities in 500-μL portions of the brain extracts and 50-μL portions of plasma extract were measured in a well counter (Canberra Packard) and corrected for decay to a common time. After decay of the [^{18}F], the samples were transferred to plastic vials, to each of which was added 10 mL of liquid scintillation counting fluid (BCS, Amersham). The total [^{3}H] activity in each vial was determined by scintillation spectroscopy (LKB Wallac).

Plasma and brain extracts were fractionated by reversed-phase HPLC as described elsewhere [8]. Arterial [^{3}H]DOPA concentrations were corrected for the presence of variable amounts of tritiated water (2–20%). Recoveries by HPLC of all labelled tracers from plasma were greater than 75% [2]. To correct for the presence of tritiated water in brain, cerebral uptake of [^{3}H]DOPA was calculated from the sum of the activities of [^{3}H]DOPA, [^{3}H]dopamine, and the tritiated acidic me-

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‡ Abbreviations: DOPA, L-3,4-dihydroxyphenylalanine; FDOPA, 6-[^{18}F]fluoro-L-DOPA; OMe-FDOPA, *O*-methyl-6-[^{18}F]fluoro-L-DOPA; OMe-[^{3}H]DOPA, *O*-methyl-[^{3}H]DOPA; COMT, catechol-*O*-methyltransferase; and PET, positron emission tomography.

tabolites in brain extracts. The purity of the FDOPA (>98%) was verified by HPLC with on-line gamma detection (Berthold). The distribution volumes of [^{3}H]DOPA and FDOPA in brain were plotted as functions of the normalized arterial integrals (θ, min^{-1}, [9–11],

$$V_d = (K_1^{DOPA} \cdot \theta) + V_p$$

where K_1^{DOPA} (mL · hg^{-1} · min^{-1}) is the unidirectional blood–brain clearance and V_p (mL · hg^{-1}) the plasma volume occupied by the tracer in brain.

For studies of peripheral tracer metabolism, rats (N = 3) were treated with carbidopa (10 mg/kg, i.p., Merck, Sharp & Dohme) 30 min prior to tracer injection as above. Arterial blood samples were collected at intervals from 0.5 to 120 min after tracer injection. The radioactivities in plasma extracts were fractionated by HPLC with successive on-line gamma detection and scintillation spectroscopy. We have described previously the integrated plasma activities of FDOPA and its *O*-methylated metabolite [12] in terms of the relative COMT activity (k_0, min^{-1}) and the elimination rate constant of the metabolite (k_{-1}, min^{-1}),

$$[\text{OMe-DOPA}]_t = k_0 \int_0^T [\text{DOPA}]_t dt - k_{-1} \int_0^T [\text{OMe-DOPA}]_t dt$$

which, upon rearrangement, yields a linear equation in terms of the integrated arterial tracer concentrations, with k_0 the ordinate intercept and k_{-1} the slope (see Fig. 1). In the present study, we calculated the *O*-methylation rates of [^{3}H]DOPA and FDOPA, and the elimination rates for the corresponding metabolites from plasma.

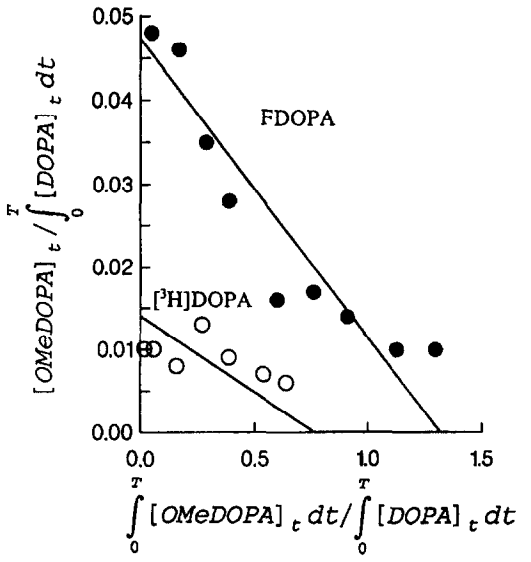

Fig. 1. Kinetic analysis of the *O*-methylation of FDOPA and [^{3}H]DOPA and the elimination rates of the respective *O*-methylated metabolites in arterial plasma of a single carbidopa-pretreated rat, expressed in terms of the integrated arterial curves of the two tracers and their metabolites. The ordinate intercepts yield the methylation rate constants (k_0) of the two tracers. The linear regression slopes correspond to the elimination rate constants of the *O*-methylated metabolites from plasma (k_{-1}).

Table 1. *O*-Methylation rate constants (k_0) and elimination rate constants of the *O*-methylated metabolites (k_{-1}) measured for [^{3}H]DOPA and FDOPA in arterial plasma of carbidopa-pretreated rats

	k_0 (min^{-1})	k_{-1} (min^{-1})
[^{3}H]DOPA	0.0095 ± 0.0016	0.0064 ± 0.0014
FDOPA	0.031 ± 0.008*	0.018 ± 0.008†

Each estimate is the mean ± SD of three separate determinations.

*,† Rate constant for FDOPA is significantly greater than for [^{3}H]DOPA: *$P < 0.01$ and †$P < 0.05$.

Rate constants and clearances for [^{3}H]DOPA and FDOPA were compared by Student's *t*-test.

RESULTS

The kinetics of the metabolism of FDOPA and [^{3}H]DOPA in plasma of the same rat are presented in Fig. 1. The mean (±SD, N = 3) coefficient of methylation for FDOPA (0.031 ± 0.008 min^{-1}) was significantly higher ($P < 0.01$) than for [^{3}H]DOPA (0.0095 ± 0.0016 min^{-1}). The elimination rate constant for OMe-FDOPA (0.018 ± 0.008 min^{-1}) was higher ($P < 0.05$) than for OMe-[^{3}H]DOPA (0.0064 ± 0.0014 min^{-1}, Table 1).

The distribution volumes of [^{3}H]DOPA and FDOPA in striatum and in parietal cortex as functions of the normalized arterial integrals are illustrated in Fig. 2. The linear estimates of the unidirectional clearances and apparent plasma volumes are reported in Table 2. The

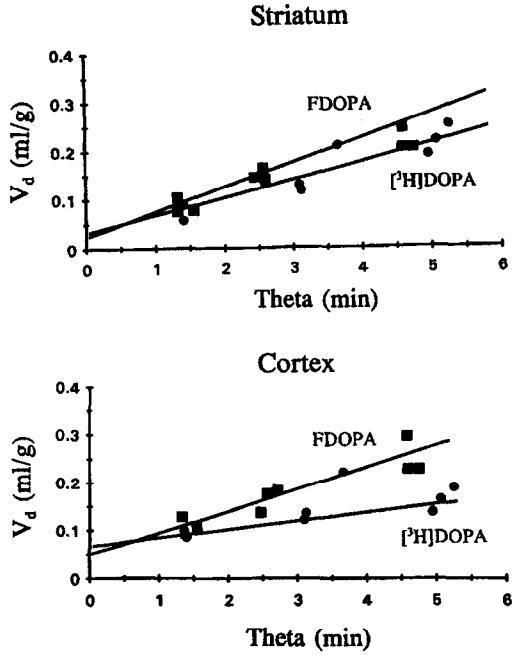

Fig. 2. Distribution volumes (V_d) of [^{3}H]DOPA (●) and FDOPA (■) in striatum and parietal cortex as functions of the normalized arterial integrals (θ, min) of the corresponding tracers. Each point is a separate determination. The linear regression slopes yield the unidirectional clearances (K_1), whereas the ordinate intercepts correspond to the apparent plasma volumes occupied by the tracers in brain.

Table 2. Summary of the unidirectional blood-brain clearances (K_1) of, and the apparent plasma volumes (V_p) for, FDOPA and [^{3}H]DOPA in rat brain

	K_1^D [^{3}H]DOPA (mL · hg^{-1} · min^{-1})	K_1^F [^{18}F]DOPA (mL · hg^{-1} · min^{-1})	V_p^D [^{3}H]DOPA (mL · hg^{-1})	V_p^F [^{18}F]DOPA (mL · hg^{-1})
Striatum	3.8 ± 0.6	4.9 ± 0.5*	2.7 ± 2.1	3.7 ± 1.6
Olfactory tubercle	3.2 ± 0.7	4.5 ± 0.6 NS	4.3 ± 2.3	5.7 ± 2.0
Parietal cortex	2.2 ± 0.7	4.4 ± 0.7*	6.7 ± 2.6	4.6 ± 2.2
Hippocampus	2.5 ± 0.9	3.4 ± 0.6 NS	4.3 ± 3.2	4.3 ± 1.9
Hypothalamus	2.6 ± 0.5	4.9 ± 0.7†	7.1 ± 1.8	4.1 ± 2.3
Cerebellum	2.7 ± 0.8	4.8 ± 0.7†	8.1 ± 2.9	6.4 ± 2.2

The unidirectional blood–brain clearances and apparent plasma volumes were measured for [^{3}H]DOPA (K_1^D, V_p^D) and for FDOPA (K_1^F, V_p^F). Each estimate is the mean ± SD of three separate estimates.

*,† $K_1^F > K_1^D$: *$P < 0.025$, and †$P < 0.01$.

‡ NS = not significant.

mean (±SD) value of K_1^D in six brain regions was 2.8 ± 0.6 mL · hg^{-1} · min^{-1}, whereas the mean value of K_1^F was 4.5 ± 0.6 mL · hg^{-1} · min^{-1}, 60% higher ($P < 0.01$). The apparent plasma volumes for [^{3}H]DOPA (5.5 ± 2.1 ml · hg^{-1}) were not consistently different from those obtained for FDOPA (4.8 ± 1.0 ml · hg^{-1}).

DISCUSSION

The present estimate of the rate constant of *O*-methylation of FDOPA (0.031 min^{-1}) was lower than an earlier estimate obtained in Long-Evans rats (0.055 min^{-1} [8]), but higher than in Sprague–Dawley rats (0.01 min^{-1} [13]). Thus, in two out of three rat studies, k_0 for FDOPA was higher than the population mean obtained in human subjects for PET studies (0.011 ± 0.002 min^{-1} [12]). Available results therefore suggest a species difference in COMT activity with respect to FDOPA, although the possibility of strain differences within rats cannot be excluded. In the present study, FDOPA was *O*-methylated more rapidly than [^{3}H]DOPA in the same rats, suggesting that ring-fluorination favors *O*-methylation *in vivo*. OMe-FDOPA was eliminated more rapidly from circulation than was OMe-[^{3}H]DOPA, suggesting that ring-fluorination may also influence renal clearance.

Phenolic acidity of catechols is enhanced greatly by the presence of fluorine in the 2- or 5-position, and to a lesser extent by fluorine in the 6-position of the aromatic ring [14, 15]. FDOPA and [^{3}H]DOPA are not kinetically distinguished by striatal-DOPA-decarboxylase *in vitro*, although 2-[^{18}F]fluoro-DOPA is a relatively poor substrate *in vivo*, due to ionization of the phenolic group *ortho* to the electron-withdrawing fluorine [16]. *In vitro* at pH 9, the affinity of 2- and 5-FDOPA for COMT is higher than that of DOPA, while 6-FDOPA has a much lower affinity than DOPA, effects also explicable on the basis of phenolic ionization [5]. The present observation that the rate constant for *O*-methylation *in vivo* of FDOPA is 3-fold higher than for [^{3}H]DOPA is not predicted by the affinities *in vitro* for COMT, although this discrepancy may be related to non-physiological pH in the COMT assays [4]. COMT may have increased access to FDOPA *in vivo* if FDOPA is transferred to liver more rapidly than is [^{3}H]DOPA. The greater elimination rate constant of OMe-FDOPA from arterial plasma (Table 1) supports the notion that the fluorinated tracers are cleared from circulation more rapidly.

Apparent plasma volumes for [^{3}H]DOPA and FDOPA in forebrain structures (~4 mL · hg^{-1}) were similar to earlier results obtained for [^{3}H]tyrosine [17]. The average value for K_1^D (4.5 mL · hg^{-1} · min^{-1}) was similar to an earlier estimate (3.7 mL · hg^{-1} · min^{-1} [13]). In four out of six brain regions examined, the unidirectional blood–brain clearances of FDOPA were higher than for [^{3}H]DOPA. The affinity of neutral amino acids for facilitated transport across the blood–brain barrier correlates with the octanol/water partition coefficient, an index of hydrophobicity [18]. The capacity factors of FDOPA and its metabolites in reversed-phase HPLC, another index of hydrophobicity, are 50% higher than for the corresponding non-fluorinated compounds [19]. The transfer of FDOPA across the blood–brain barrier may be facilitated by intrinsic hydrophobicity of the molecule. Thus, ring fluorination of DOPA alters tracer disposition with respect to peripheral metabolism and blood–brain permeability.

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