Biochemical Pharmacology, Vol. 37, No. 2, pp. 247–250, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

KINETICS OF *IN VITRO* DECARBOXYLATION AND THE *IN VIVO* METABOLISM OF 2-^{18}F- AND 6-^{18}F-FLUORODOPA IN THE HOODED RAT

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(*Received* 26 *February* 1987; *accepted* 3 *June* 1987)

Abstract—The metabolisms of L-^{18}F-2-fluoro-DOPA (L-2-^{18}F-DOPA) and L-^{18}F-6-fluoro-DOPA (L-6-^{18}F-DOPA) were compared *in vitro* and *in vivo* in the carbidopa-pretreated male hooded rat. *In vivo*, the sole metabolites in plasma were O-methylated derivatives. The peripheral formation of the O-methylated derivative of L-2-^{18}F-DOPA was approximately twice as great as that for the 6-isomer. Animals were killed at 10 and 60 min after administration of the ^{18}F-DOPAs, and samples of striatum and vermis were analyzed by HPLC. L-2-^{18}F-DOPA passed less readily into brain than did L-6-^{18}F-DOPA. Whereas significant amounts of 6-^{18}F-fluorodopamine and metabolites were formed in the striatum, no decarboxylated derivatives of L-2-^{18}F-DOPA were found. Determination of the Michaelis–Menten kinetic constants for aromatic amino acid decarboxylase (AADC, EC 4.1.1.26) indicated that the K_m for L-2-^{18}F-DOPA (982 ± 115 μM) was considerably higher than that for L-6-^{18}F-DOPA (101 ± 22 μM). The low substrate affinity for AADC and the relatively more rapid rate of O-methylation in the periphery account for the lack of formation of 2-^{18}F-fluorodopamine *in vivo*. The ratio of total radioactivity between striatum and other brain regions is related to the decarboxylation of radiolabeled tracer and the relative persistence of the decarboxylated derivatives in the striatum. Since L-2-^{18}F-DOPA is not decarboxylated in rat striatum, it is not a promising agent for the study of cerebral DOPA metabolism in humans by means of positron emission tomography.

The advent of positron emission tomography (PET) has permitted the study of cerebral metabolism in humans by non-invasive means. Although the metabolism of [^{11}C]D,L-DOPA has been studied in experimental animals [1], the majority of research directed toward the development of PET tracers for cerebral DOPA metabolism has been conducted with ^{18}F-labelled derivatives of L-DOPA. It was hoped that the ring-fluorinated analogs would behave similarly to L-DOPA and its metabolites. The three possible ring-fluorinated ^{18}F-fluorodopamines have been shown to compete with ligands binding to D_1 and D_2 dopamine binding sites [2]. An accumulation of radioactivity in the striatum is observed following the administration of L-6-^{18}F-DOPA to Cynomolgus monkeys [3] or to conscious humans [4]. The ratio of radioactivity (radiocontrast) obtained with L-6-^{18}F-DOPA between the striatum and other brain regions is reduced in humans exposed to *N*-methyl-*p*-phenyl-tetrahydropyridine (MPTP) [5] and in cases of idiopathic Parkinsonism [6]. These results suggest that the radiocontrast observed in PET studies using L-6-^{18}F-DOPA is related to the entry of L-6-^{18}F-DOPA into the pathway for dopamine metabolism in the striatum. Previous work in carbidopa-pretreated male hooded rats [7] has indicated that ^{18}F-6-fluorodopamine (^{18}F-DA) and ^{18}F-6-fluoro-dihydroxyphenylacetic acid (^{18}F-DOPAC)) are major constituents of radioactivity in rat striatum, while ^{18}F-6-fluoro-3-*O*-methylDOPA (OMe-^{18}F-DOPA) is the only significant metabolite in other brain areas and in plasma. The only published report on the kinetics of the decarboxylation of an ^{18}F-DOPA was conducted with 5-fluoro-D,L-DOPA [8]. Therefore, experiments were conducted in order to determine the kinetic constants for the decarboxylation of L-2- and L-6-^{18}F-DOPA. In addition, the metabolisms in brain and in the periphery of the male hooded rat were compared for the two compounds.

MATERIALS AND METHODS

Carbidopa was provided by Merck, Sharp & Dohme. L-DOPA, pargyline hydrochloride and pyridoxal phosphate were obtained from Sigma. D,L-6-FluoroDOPA was synthesized [9], and positron emitting L-2-^{18}F-DOPA and L-6-^{18}F-DOPA were prepared simultaneously and separated as described elsewhere [10]. The two compounds were produced on each day of use, and the activities obtained were in a ratio of L-2-^{18}F-DOPA/L-6-^{18}F-DOPA = 0.80 ± 0.02 (N = 4). The specific activity of each was 175–230 Ci/mole at the end of synthesis.

The concentration of the L-6-^{18}F-DOPA solution obtained from the preparative procedure was determined to be 2.5 ± 0.2 mM by ultraviolet absorption

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(Unican SP-500 Spectrophotometer, $\varepsilon 283 = 3836$ l/mole-cm) with D,L-6-fluoroDOPA serving as the standard. Since no standard was available, the concentrations of the L-2-^{18}F-DOPA solutions ($\varepsilon 271 = 1850$ l/mole-cm) were calculated indirectly, given the daily L-6-^{18}F-DOPA concentration and the relative synthetic yields of the two molecules. The extinction coefficient determined for L-DOPA ($\varepsilon 280 = 2648$ l/mole-cm) agreed closely with the literature value [11].

For the determination of the kinetic constants for decarboxylation, triplicate samples of L-2- and L-6-^{18}F-DOPA at final concentrations ranging from 50–500 μM were incubated for 30 min at 37°. The medium contained 25 μl of freshly prepared striatal homogenate (10:1 in 0.5% Triton-X), 100 μM pargyline, 50 μg pyridoxal phosphate and phosphate buffer (100 mM, pH 7.2) in a final volume of 250 μl. Reactions were terminated by the addition of 250 μl of 0.1 M perchloric acid. After centrifugation for 20 min at 10,000 g, 20-μl samples were analyzed by reversed phase HPLC at a flow rate of 1.25 ml/min with gamma counting of fractions as described previously [7]. The retention times and capacity factors of the various compounds are indicated in Table 1. Kinetic constants are reported as mean ± SE of three separate determinations.

The amount of radioactivity in each ^{18}F-DA peak was measured in a gamma counter (Packard Auto-Gamma 5650) and used to calculate the amount of decarboxylation. Incubations in which the enzyme was omitted indicated that L-2-^{18}F-DOPA, but not L-6-^{18}F-DOPA, was non-enzymatically decarboxylated at a rate of 0.8%/hr. Therefore, rates determined for 2-^{18}F-DA formation were corrected by subtraction of the blank value. Recovery of radioactivity by HPLC was essentially quantitative.

In a separate series of experiments the K_m for D,L-DOPA and the K_i for D,L-6-F-DOPA were determined by measuring the amount of $^{14}CO_2$ formed on incubation of rat striatal homogenates with [1-^{14}C]-D,L-DOPA (New England Nuclear) at seven concentrations ranging from 30 to 400 μM in the presence of 0–900 μM D,L-6-F-DOPA (six concentrations used). The assay was done as previously described [12], and the kinetic constants were calculated by the method of Dixon [13].

Protein content of the striatal homogenates was determined by the Bradford assay [14].

For *in vivo* studies of the metabolism of the L-^{18}F-DOPAs, male hooded rats (250–350 g) were provided with intrajugular cannulae while under chloropent anesthesia. After recovery, animals were pretreated with carbidopa (5 mg/kg, i.p.) 30 min prior to administration of either L-2- or L-6-^{18}F-DOPA (500 μCi/kg) via the cannula. Blood samples were taken at intervals between 5 and 90 min. Plasma was separated by centrifugation for 5 min at 5000 g and deproteinized by the addition of an equal volume of 0.25 M perchloric acid. After 30 min on ice, samples were centrifuged for 30 min at 10,000 g, and 100-μl samples of supernatant were fractionated by HPLC. Animals were killed at 10 and 60 min after L-^{18}F-DOPA injection. Samples of striatum and vermis were homogenized in 10 vol. of 0.1 M perchloric acid and centrifuged for 30 min at 10,000 g. Aliquots (200 μl) of each extract were fractionated by HPLC. The radioactivity in each chromatographic peak was determined by gamma counting of fractions and was corrected to the time of injection of the animal (^{18}F half-life = 109.7 min).

Table 1. Retention times and capacity factors in the HPCL system for some fluorinated compounds

	Retention time (min)	Capacity factor* (k')
L-2-^{18}F-DOPA	3.2	0.6
2-^{18}F-DA	10.4	4.2
L-6-^{18}F-DOPA	3.9	1.0
6-^{18}F-DA	13.5	5.8

* The capacity factor k' is calculated as $= V_r/V_m - 1$, where V_r is the retention volume and V_m is the volume of the mobile phase.

RESULTS

The results of typical assays for aromatic amino acid decarboxylase (AADC, EC 4.1.1.26) with L-2- and L-6-^{18}F-DOPA serving as the substrates are illustrated in Fig. 1. Each point represents the mean of two determinations, with variability being less than 15%. The K_m determined for L-2-^{18}F-DOPA was 982 ± 115 μM (N = 3) and the V_{max} was 110 ± 10 nmol · (mg protein)$^{-1}$ · hr^{-1}. The K_m determined for L-6-^{18}F-DOPA was 101 ± 22 μM (N = 3) and the V_{max} was 180 ± 20 nmol · (mg protein)$^{-1}$ · hr^{-1}.

The studies using radioactive [^{14}C]-D,L-DOPA and cold D,L-6-F-DOPA indicated competitive inhibition, and the K_m and K_i values determined were, respectively, 228 ± 7 and 428 ± 57 μM (N = 3).

The results of *in vivo* experiments are illustrated in Table 2. The average recovery of radioactivity by HPLC was 88 ± 2% in the case of brain extracts and 90 ± 3% from plasma extracts. In contrast to animals receiving L-6-^{18}F-DOPA, no ^{18}F-fluorodopamine or other decarboxylated metabolite was detected in striatal samples from animals receiving L-2-^{18}F-DOPA;

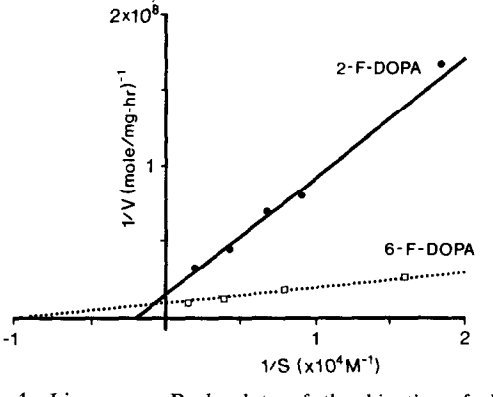

Fig. 1. Lineweaver–Burk plots of the kinetics of the decarboxylation of L-2-^{18}F- and L-6-^{18}F-DOPA by the same striatal homogenate. Enzyme velocity (V) was calculated in units of nmol ^{18}F-dopamine formed per mg protein per hr. Each point represents the mean of two determinations which differed by less than 15%.

Table 2. Radiochemical composition of various tissues as a function of time after administration of either L-2-^{18}F- or L-6-^{18}F-DOPA in the hooded rat

		L-2-^{18}F-DOPA			L-6-^{18}F-DOPA†		
Time	Tissue*	Total activity	^{18}F-DOPA	OMe-^{18}F-DOPA	Total activity	^{18}F-DOPA	OMe-^{18}F-DOPA
At 10 min	Striatum	276 ± 31 (4)	22 ± 4	217 ± 4	580 ± 83 (5)	71 ± 4	182 ± 10
	Vermis	416 ± 54 (4)	25 ± 3	379 ± 4	612 ± 86 (5)	183 ± 11	272 ± 20
	Plasma	1089 ± 31 (4)	175 ± 4	870 ± 20	888 ± 75 (8)	328 ± 15	402 ± 21
	S/V	0.68 ± 0.06 (4)			0.94 ± 0.02 (5)		
At 60 min	Striatum	281 ± 23 (3)	4 ± 1	256 ± 3	601 ± 51 (8)	18 ± 4	312 ± 3
	Vermis	367 ± 47 (3)	4 ± 2	340 ± 9	336 ± 24 (8)	20 ± 2	291 ± 15
	Plasma	514 ± 20 (3)	78 ± 10	409 ± 9	558 ± 36 (8)	48 ± 11	420 ± 15
	S/V	0.78 ± 0.04 (3)			1.78 ± 0.05 (8)		

* Activity, corrected for decay to the time of injection, is reported as cpm/mg in the case of brain tissues and cpm/μl in the case of plasma. All values are reported as the mean ± SEM of (N) determinations. The ratio S/V refers to the ratio of total activity in striatum and vermis.

† In addition, the striatal extracts of L-6-^{18}F-DOPA-treated animals contained ^{18}F-DA, ^{18}F-DOPAC, ^{18}F-HVA, and ^{18}F-6-fluoro-3-methoxytyramine with the total radioactivity in these materials being about 33% of the total at both time periods [7]; none of these decarboxylated metabolites was seen in the vermis or plasma, or in L-2-^{18}F-DOPA-treated animals.

the limits of detection were such that this means striatal L-2-^{18}F-DA levels must have been less than 10% of striatal L-6-^{18}F-DA concentrations. The ratio of total activity in striatum to that in vermis remained less than one in animals with L-2-^{18}F-DOPA over a period during which radiocontrast developed in the animals with L-6-^{18}F-DOPA. The ratio of ^{18}F-DOPA activity in plasma to ^{18}F-DOPA in vermis was considerably higher in animals receiving L-2-^{18}F-DOPA than in animals with L-6-^{18}F-DOPA. In contrast, the partitioning of OMe-^{18}F-DOPA between plasma and vermis was approximately equal for both L-2- and L-6-^{18}F-DOPA.

Both the disappearance of ^{18}F-DOPA from plasma and the accumulation of OMe-^{18}F-DOPA occurred more rapidly with the L-2- than the L-6-isomer. Biexponential disappearance from plasma of both total radioactivity (λ_1 and λ_2) and of ^{18}F-DOPA activity (λ_3 and λ_4), previously noted in carbidopa-pretreated humans [15] and rats [7] receiving L-6-^{18}F-DOPA, was also observed in rats receiving L-2-^{18}F-DOPA. Regression analysis of OMe-^{18}F-DOPA activity in plasma at late time points yielded a time constant (λ_5) presumably related to renal elimination of this metabolite. The calculated half-lives for L-2- and L-6-^{18}F-DOPA metabolism are compared in Table 3. Extrapolation to T_0 of the lines of regression for the amounts of total ^{18}F, ^{18}F-DOPA and OMe-^{18}F-DOPA in plasma as a function of time suggested that approximately 49% of the L-2-^{18}F-DOPA and only 23% of the L-6-^{18}F-DOPA was rapidly converted to the methylated derivatives in the periphery.

DISCUSSION

The position of the fluorine on the aromatic ring has potent effects on the disposition and metabolism of DOPA analogs. With respect to positron emission tomography, the most significant finding was that L-2-^{18}F-DOPA was a poor substrate for AADC *in vitro* and was not decarboxylated in rate striatum. In contrast, the K_m determined for L-6-^{18}F-DOPA (100 μM) fell within the range of literature values for L-DOPA (190 μM [16], 200 μM [17], 40 μM [18]) and was consistent with the K_m (228 μM) we obtained for D,L-DOPA on the assumption that the D-DOPA is inert. The K_i value obtained for D,L-6-F-DOPA, taken together with the K_m values determined for D,L-DOPA and L-6-^{18}F-DOPA, suggest that the L-DOPA and L-6-F-DOPA may behave as almost equivalent substrates for the enzyme. Similarly, the K_m reported for D,L-5-^{18}F-DOPA was close to that determined by the same group for DOPA [8].

Since radiocontrast between the striatum and

Table 3. Half-lives for the disappearance of total radioactivity, ^{18}F-DOPAs and OMe-^{18}F-DOPAs from plasma of carbidopa-pretreated rats

	Total ^{18}F activity		^{18}F-DOPA		OMe-^{18}F-DOPA
	T_1	T_2	T_3	T_4	T_5
L-6-^{18}F-DOPA	7.9 ± 0.7	350 ± 100	3.3 ± 0.2	34 ± 2	∞
L-2-^{18}F-DOPA	4.8 ± 0.8	90 ± 10	2.9 ± 0.5	110 ± 15	66 ± 5

Decay constants, calculated as described in the text, are here reported as half-lives in min where $T = t_{1/2} = \ln 2/\lambda$. The persistence of OMe-6-^{18}F-DOPA in plasma was too long to permit estimation of the elimination rate.

other brain regions in experiments with L-6-^{18}F-DOPA is related to the formation of 6-^{18}F-fluorodopamine [7], the present findings indicate that radiochemical purity is essential for interpretation of PET data.

The rapid O-methylation of L-2-^{18}F-DOPA is in agreement with studies comparing the affinities of ring-fluorinated DOPAs for catechol-*O*-methyltransferase (COMT, EC 2.1.1.6) [19]. About half of the injected L-2-^{18}F-DOPA seems to be rapidly O-methylated in the periphery. The resulting reduced availability of plasma L-2-^{18}F-DOPA may be a contributing factor in the lack of formation of 2-^{18}F-fluorodopamine in rat striatum. An additional factor may be relatively poor transport of L-2-^{18}F-DOPA into brain tissue.

The numerous differences between the metabolism of L-2- and L-6-^{18}F-DOPA may all be related to differences in the electron withdrawing strength of a fluorine atom in different positions on an aromatic ring. A 5-fluoro substituent is reported to direct O-methylation on to the 4-hydroxy group of DOPA [19] or noradrenaline [20], unlike most endogenous substrates for COMT which are methylated in the 3-position [21, 22]. Thus, for the three possible ring-fluorinated DOPAs, it is observed that the position of O-methylation of the catechol system is correlated with the presence of a fluorine atom either *ortho* or *para* to the phenolic hydroxyl group which is methylated. This is thought to be a result of enhanced acidity of the phenolic hydroxyl in those systems [19, 20]. In consequence, one would predict that both L-2- and L-6-^{18}F-DOPA should be activated for O-methylation in the 3-position, with the more potent *in vivo* O-methylation of L-2-^{18}F-DOPA being attributable to the closer proximity of the activating and reacting groups.

The presence of a hydroxyl group in the 3-position of the aromatic ring contributes to the ability of a variety of compounds to inhibit AADC competitively [23, 24], presumably because of interaction between the phenolic group and the co-factor, pyridoxal phosphate. Compounds lacking a 3-hydroxy group, including compounds with a 3-methoxy group, have relatively low affinities for AADC. Increased 3-phenolic ionization produced by an adjacent fluorine atom may be sufficient explanation for the low affinity of L-2-^{18}F-DOPA for AADC. The concentration of L-2-^{18}F-DOPA in striatum following intravenous injection is presumably so low as to forbid the formation of amounts of 2-^{18}F-fluorodopamine detectable by either HPLC or PET.

Acknowledgements—This research was sponsored by the Medical Research Council of Canada and by the Health Care Research Foundation of British Columbia. W. R. W. Martin is an MRC scholar. The authors wish to thank Ms. Salma Jivan for assistance in the preparation of the radiochemicals.

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