Biochemical Pharmacology, Vol. 39, No. 12, pp. 1853–1860, 1990.
Printed in Great Britain.

0006-2952/90 $3.00 + 0.00

COMPARATIVE *IN VIVO* METABOLISM OF 6-[^{18}F]FLUORO-L-DOPA AND [^{3}H]L-DOPA IN RATS

WILLIAM P. MELEGA, ANDRÉ LUXEN, MILTON M. PERLMUTTER, CHARNA H. K. NISSENSON, MICHAEL E. PHELPS and JORGE R. BARRIO*

Division of Nuclear Medicine and Biophysics, Departments of Radiological Sciences and Pharmacology, UCLA School of Medicine and The Laboratory of Nuclear Medicine, Los Angeles, CA 90024, U.S.A.

(*Received* 29 *June* 1989; *accepted* 20 *September* 1989)

Abstract—*In vivo* double-label experiments in rats were designed to correlate the peripheral and cerebral metabolism of 6-[^{18}F]fluoro-L-DOPA ([^{18}F]FDOPA) with that of [^{3}H]L-DOPA. Authentic samples of the major [^{18}F]FDOPA metabolites were synthesized to identify the ^{18}F-labeled metabolites. After carbidopa pretreatment and intravenous administration of the compound, the products of peripheral metabolism in plasma were analyzed at times from 3 to 60 min. In the periphery, amine conjugates were detected but they accounted for less than 15% of the total radioactivity; the major metabolites were 3-*O*-methyl-6-[^{18}F]fluoro-L-DOPA and 3-*O*-methyl-[^{3}H]L-DOPA. The rate and extent of 3-O-methylation of [^{18}F]FDOPA exceeded that of [^{3}H]L-DOPA. Both 3-O-methylated products entered the striatum and cerebellum where they contributed significant but uniform activity. Analysis of cerebral metabolism in these structures indicated a linear accumulation of total radioactivity: a striatum/cerebellum ratio of 2 was observed by 60 min. 6-[^{18}F]Fluorodopamine (35%) and [^{3}H]dopamine (55%) were the major metabolites formed in the striatum: however, the methylated [^{18}F]FDOPA and [^{3}H]DOPA products of predominantly peripheral origin represented 55% (^{18}F) and 35% (^{3}H) of the total radioactivity respectively. Other [^{3}H]dopamine metabolites and their ^{18}F-labeled analogs represented less than 10–15% at all times analyzed. The cerebellum radioactivity was composed only of [^{18}F]FDOPA, [^{3}H]DOPA and their 3-O-methylated products. These data will serve as the basis for the development of kinetic models of [^{18}F]FDOPA metabolism that can be applied to the evaluation of central dopamine biochemistry with positron emission tomography in humans.

6-[^{18}F]Fluoro-L-DOPA ([^{18}F]FDOPA) is a biochemical probe used for the *in vivo* assessment of presynaptic dopaminergic function with positron emission tomography (PET) [1, 2]. As an L-DOPA analog tracer, [^{18}F]FDOPA should follow the metabolic pathway of L-DOPA (Fig. 1). Accordingly, in the periphery, [^{18}F]FDOPA would be subject to either extensive 3-O-methylation or decarboxylation and subsequent conjugation. In the brain, [^{18}F]FDOPA striatal accumulation and subsequent decarboxylation to 6-fluorodopamine ([^{18}F]FDA) followed by oxidative deamination and 3-O-methylation would occur. Our preliminary experiments, in fact, indicated these pathways to be operative, however, previous studies on the peripheral and cerebral metabolism of [^{18}F]FDOPA in rats [3], monkeys [4, 5], and humans [6] have produced inconsistent results with their respective [^{18}F]FDOPA metabolite profiles. Especially noteworthy are the apparent contradictory data produced to establish the presence of 3-*O*-methyl-6-[^{18}F]fluoro-L-DOPA ([^{18}F]3-OMFD) as [3], or as not [4–6], the major metabolite of [^{18}F]FDOPA. Obviously, this issue requires clarification because the development of kinetic models of [^{18}F]FDOPA metabolism for PET is totally dependent on accurate, time-dependent [^{18}F]FDOPA metabolite profiles. Such profiles can then be used to develop a correlation between [^{18}F]FDOPA kinetics and endogenous dopamine metabolism.

To address the issue of metabolite identification, in this work we synthesized the anticipated major metabolites of [^{18}F]FDOPA [7]. To evaluate the assumption that [^{18}F]FDOPA cerebral metabolism reflects that of the endogenous dopamine system, we designed double-label experiments in which the metabolism of [^{18}F]FDOPA could be assayed and compared with that of [^{3}H]L-DOPA ([^{3}H]DOPA), a tracer used extensively to assess the metabolism of the endogenous dopamine system. The results of these experiments will provide the necessary data not only for the development of accurate [^{18}F]FDOPA kinetic models, but for determination of the relevancy of [^{18}F]FDOPA kinetics and metabolism to endogenous cerebral dopamine metabolism.

MATERIALS AND METHODS

Chemicals. Monobasic sodium phosphate, 1-octanesulfonic acid (OSA) sodium salt, and arylsulfatase (Type VI, prepared from *Aerobacter aerogenes*) were purchased from Sigma Chemical; perchloric acid (70% aqueous solution) and HPLC optima-grade methanol were purchased from Fisher; and sodium metabisulfite and EDTA disodium salt from EM Science. Ecoscint was from National Diagnostics, and L-3,4-[2,5,6-^{3}H]dihydroxyphenylalanine (sp. act. 40 Ci/mmol) from New England Nuclear. 6-[^{18}F]Fluoro-L-DOPA (sp. act. 1–2 Ci/mmol) was prepared by using the radiofluorodemercuration procedure previously described [7].

* Send reprint requests to: Jorge R. Barrio, Ph.D., Division of Nuclear Medicine and Biophysics, UCLA School of Medicine, Los Angeles, CA 90024.

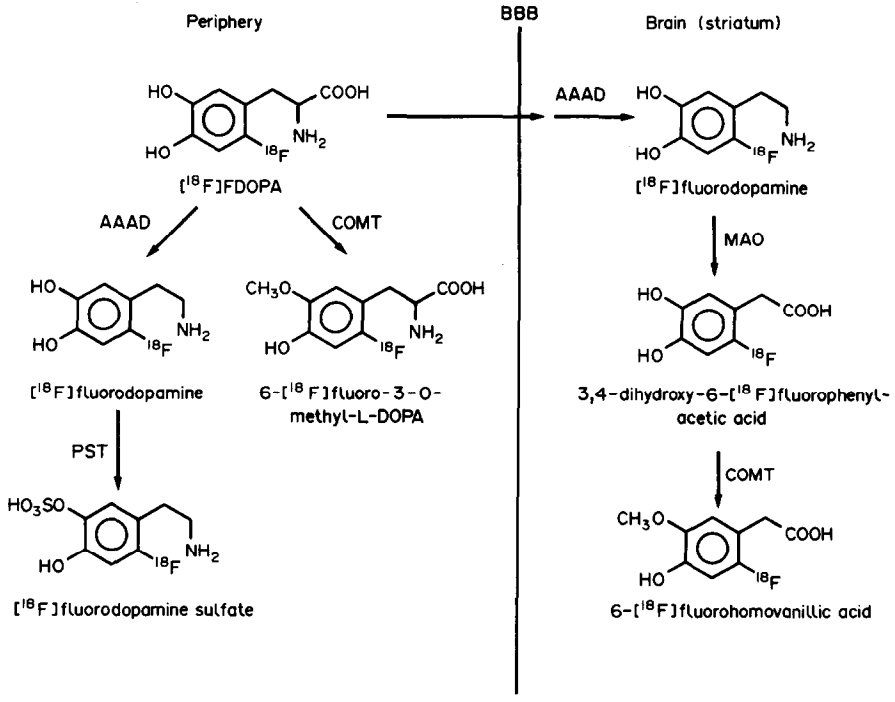

Fig. 1. Characterization of the major metabolites of [^{18}F]DOPA in the periphery and in the striatum. Abbreviations: AAAD (aromatic L-amino acid decarboxylase), COMT (catechol-*O*-methyltransferase), PST (phenolsulfotransferase), MAO (monoamine oxidase), and BBB (blood–brain barrier).

The same method was extended [8] to the synthesis and characterization of the major fluoro-L-DOPA metabolites, namely, 3,4-dihydroxy-6-fluorophenylacetic acid, 6-fluoro-3-*O*-methyl-DOPA (3-OMFD), 6-fluorodopamine and 6-fluorohomovanillic acid. Since much of our discussion focuses on the biochemical characterization of [^{18}F]3-OMFD, the synthetic route and ^{1}H NMR of 3-OMFD are presented. The synthesis of 3-OMFD was achieved from commercially available 3-*O*-methyl-L-tyrosine via a six-step reaction sequence which included the following reactions: (1) esterification, (2) trifluoroacetamidation, (3) trimethylacetylation, (4) regioselective mercuration, (5) regioselective fluorodemercuration, and (6) alkaline hydrolysis. ^{1}H NMR (DCl/DSS): δ 3.22–3.39 (m, 2H, CH_2), 3.85 (s, 3H, OCH_3), 4.39 (m, 1H, CH), 6.78 (d, 1H, $^3J_{5,F}$ = 10.5 Hz, H-5), and 6.96 (d, 1H, $^4J_{2,F}$ = 7.0 Hz, H-2).

The identity of the 6-fluorodopamine sulfate was tentatively established by two methods, at 10 min post injection from a [^{18}F]FDOPA experiment: (a) a perchloric acid plasma extract was obtained and heated at 90° for 20 min, a treatment used to hydrolyze dopamine sulfate [9], and (b), a 100-μL plasma fraction was obtained and incubated with 25 mUnits of sulfatase for 60 min at 37° [10]. In each instance, HPLC analysis indicated that the tentative conjugate radioactivity peak was predominantly shifted to the retention time corresponding to 6-fluorodopamine.

Carbidopa was a gift of Merck, Sharp & Dohme. All buffers were prepared with 0.22-μm filtered Milli-Q reagent grade water (Millipore).

Central and peripheral metabolism in rodents. Male Sprague–Dawley rats (Banton & Kingman, Freemont, CA) (275–325 g) were anesthetized with halothane and provided with intrajugular cannulae. The cannulae were heparinized and passed through the back of the neck. The animals were allowed to recover and were housed individually for 1–2 days. On the day of the experiment (injection time between 9:30 and 11:00 a.m.) the animals were pretreated with carbidopa (5 mg/kg, s.c.) 60 min before injection (10–20 sec) of [^{18}F]FDOPA [800 μCi/kg (0.4 to 0.8 μmol/kg) i.v.] and [^{3}H]DOPA [150 μCi/kg (3.8 nmol/kg) i.v.] via the cannulae in a volume of 0.4 to 0.8 mL.

Blood samples were withdrawn from the cannula for peripheral metabolite analysis at 3, 10, 15, 30 and 60 min; for brain tissue metabolite analysis, the animals were killed by decapitation at 15, 30, and 60 min. Heparinized blood samples were centrifuged in an Eppendorf microfuge at 4° and the plasma was removed. To the plasma was added an equal volume of a cold solution containing 0.8 M perchloric acid, 1.0% $Na_2S_2O_5$, 0.1% EDTA; the resulting suspension was centrifuged and then filtered through a 0.2-μm filter before HPLC analysis. The extraction of plasma [^{18}F] and [^{3}H] radioactivity was essentially quantitative (95–100%); the values reported remain uncorrected. The extraction of [^{18}F]FDOPA and [^{3}H]DOPA metabolites from striatum and cerebellum was greater than 85%; the values reported also remain uncorrected.

The striata were dissected rapidly on an ice-chilled aluminum plate, blotted, and immersed in liquid

nitrogen (within 3 min), then weighed and counted for total [^{18}F] activity. The tissues were homogenized in 0.5 mL of a solution containing 0.4 M perchloric acid, 0.5% $Na_2S_2O_5$ and 0.05% EDTA, centrifuged, and filtered through a 0.2-μm filter before HPLC analysis.

Analytical methods. The HPLC system consisted of a Beckman 210 solvent delivery system with the following mobile phase: 80% 0.1 M NaH_2PO_4, 2.6 mM OSA, 0.1 mM EDTA, pH 3.1; 20% MeOH at a flow rate of 1 mL/min. A guard cell (+0.45 V, ESA Inc.) preceded the Rheodyne 7125 injector fitted with a 100-μL loop. A guard column (30 mm × 4.6 mm, 5 μm; RP-18, Brownlee) was connected to the C_{18} reverse phase column (250 mm × 4.6 mm, 5 μm; Beckman) which was followed by a 0.2-μm carbon filter (ESA). The analytical cell (model 5011, ESA) was set at an applied potential of +0.02 V at detector 1 and +0.40 V at detector 2 and coupled to a coulochem controller (model 5100A, ESA) to monitor the levels of endogenous catecholamines throughout the experiment. Fractions (1 mL/min) were collected by an automated fraction collector (Gilson FC-80K) and counted for radioactivity (30 sec) in a gamma counter. Background activity was subtracted, and the samples were decay-corrected back to the time of injection. The [^{18}F] activity was allowed to decay (24 hr), and then a 0.8-mL aliquot of each fraction was placed in a scintillation vial containing 4 mL of Ecoscint and its [^{3}H] radioactivity counted. Both [^{18}F] and [^{3}H] peak radioactivities were identified by comparison of their retention times with those of authentic metabolite standards. The results are expressed as (a) peak percent of total radioactivity for either the plasma or striatum samples, or (b) radioactivity counts per gram weight tissue.

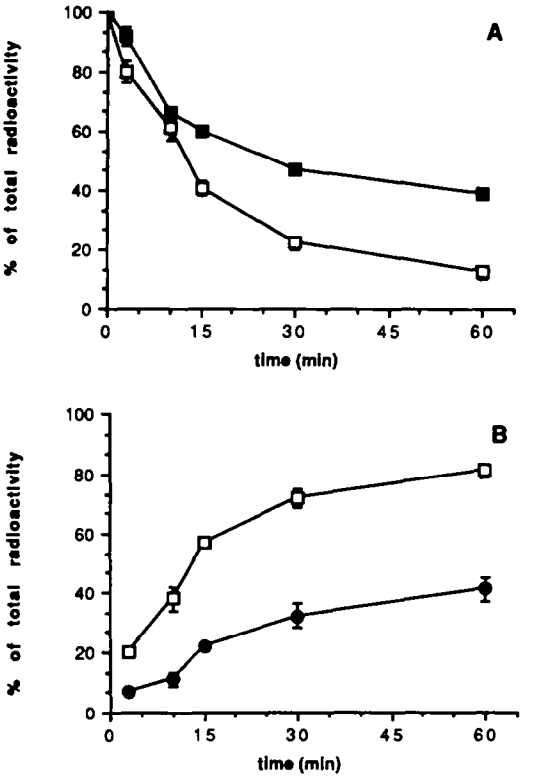

Fig. 2. (A) Clearance of [^{18}F]FDOPA (□) and [^{3}H]L-DOPA (■) from plasma. (B) Plasma levels of [^{18}F]3-OMFD (□) and [^{3}H]3-OMD (●) after double-label injection of [^{18}F]FDOPA and [^{3}H]L-DOPA. Animals were pretreated with carbidopa (5 mg/kg, s.c.) 60 min before injection (10–20 sec) of [^{18}F]FDOPA [800 μCi/kg (0.4 to 0.8 μmol/kg) i.v.] and [^{3}H]DOPA [150 μCi/kg (3.8 nmol/kg) i.v.]. Each data point is the mean ± SD from values of three to five animals.

RESULTS

Metabolites of [^{18}F]DOPA in plasma. The plasma levels of [^{18}F]FDOPA and [^{3}H]DOPA declined rapidly following intravenous bolus injection of the tracers but the relative plasma level of [^{3}H]DOPA remained higher than that of [^{18}F]FDOPA up to 60 min post injection (Fig. 2A). The major peripheral metabolites of both [^{18}F]FDOPA and [^{3}H]DOPA were [^{18}F]3-OMFD and 3-*O*-methyl-[^{3}H]L-DOPA, ([^{3}H]3-OMD), respectively, from 3 to 60 min post injection; by 60 min, the [^{18}F]3-OMFD level was 80% of the total radioactivity, whereas the [^{3}H]3-OMD level was only 40% of the total (Fig. 2B). The relative rate of 3-O-methylation for [^{18}F]FDOPA versus [^{3}H]DOPA was measured as the [^{18}F]3-OMFD/[^{18}F]DOPA versus [^{3}H]3-OMD/[^{3}H]DOPA ratio. From 30 to 60 min, the [^{18}F] ratio was six times higher than the [^{3}H] ratio (data not shown).

The metabolite profile, as shown in a representative HPLC chromatogram at 30 min (Fig. 3A), indicated that, in the presence of carbidopa, the peripheral metabolism of [^{18}F]FDOPA was essentially restricted to [^{18}F]3-OMFD; a fluoroconjugate, tentatively identified as fluorodopamine sulfate, was less than 10% of the total radioactivity. 6-[^{18}F]Fluorodopamine ([^{18}F]FDA) and 3,4-dihydroxy-6-[^{18}F]fluorophenylacetic acid ([^{18}F]-FDOPAC) were not detected at any time points (Fig. 3A).

Cerebral metabolism of [^{18}F]FDOPA. Both the [^{18}F] and [^{3}H] striatum/cerebellum (s/c) total radioactivity ratios increased linearly between 15 and 60 min post injection (Fig. 4). At 60 min, an s/c ratio of approximately 2 was obtained for both isotopes.

HPLC analysis of the radioactivity (representative chromatogram in Fig. 3B) in the striatum indicated extensive metabolism of [^{18}F]FDOPA to [^{18}F]FDA and of [^{3}H]DOPA to [^{3}H]dopamine ([^{3}H]DA) (Fig. 5, A and B); however, the [^{18}F]FDA and [^{3}H]DA levels (counts/g) decreased by only 20% between 15 and 60 min. The mean [^{18}F]FDA levels (counts/g striatum)/(mCi injected/kg animal mass) decreased from a value of 5.1×10^4 at 15 min to 4.1×10^4 at 60 min, and [^{3}H]DA levels from 2.0×10^5 at 15 min to 1.5×10^5 at 60 min. As a percent of the total striatal activity, the [^{3}H]DA level (50–55%) remained higher than that of [^{18}F]FDA (35–40%). Other [^{18}F]FDA metabolites, namely [^{18}F]FDA-sulfate, [^{18}F]FDOPAC and 6-[^{18}F]fluorohomovanillic acid ([^{18}F]FHVA) and the corresponding [^{3}H]DA

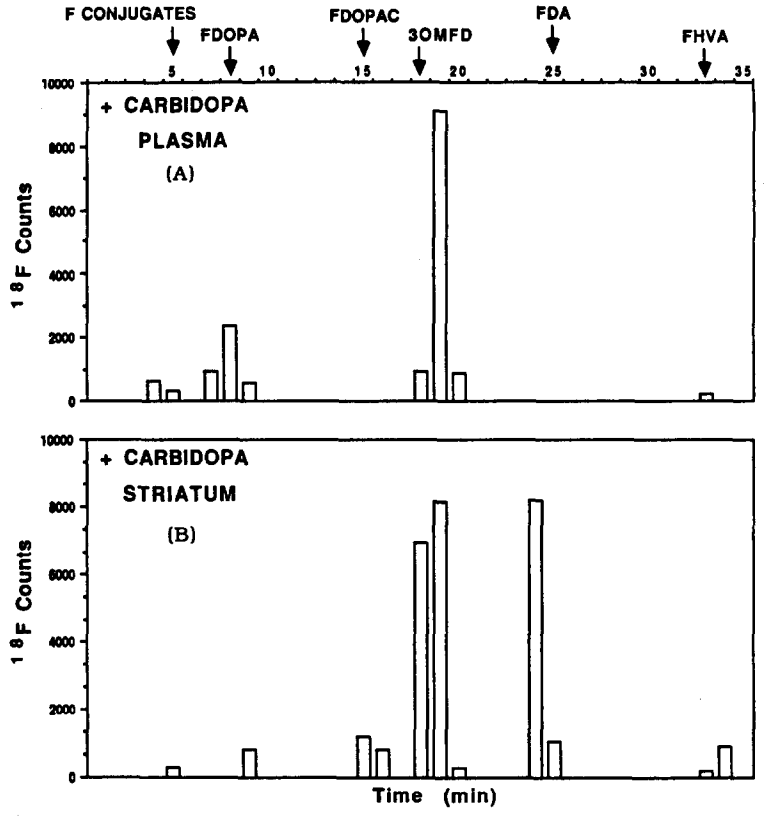

Fig. 3. Representative HPLC chromatogram profiles of radioactivity 30 min after [^{18}F]FDOPA injection. The retention times of the authentic, unlabeled fluorometabolites are indicated by the arrows. (A) Plasma radioactivity. No [^{18}F]FDOPAC or [^{18}F]FDA was detected. (B) Striatum radioactivity. (See Materials and Methods for analytical details.)

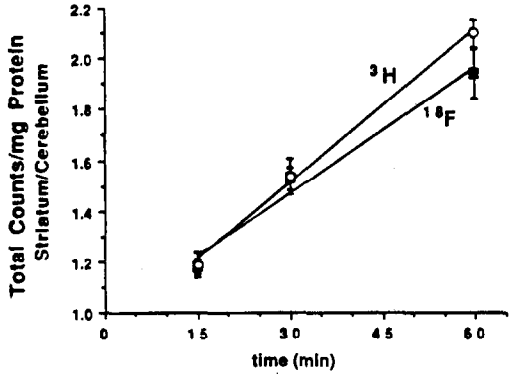

Fig. 4. Total [^{18}F] (■) and [^{3}H] (○) radioactivity for the striatum/cerebellum ratio. Each data point is the mean ± SD from values of three to five animals.

metabolites, [^{3}H]DA sulfate, [^{3}H]3,4-dihydroxyphenyl acetic acid ([^{3}H]DOPAC), and [^{3}H]homovanillic acid ([^{3}H]HVA) did not individually exceed 10% of the total radioactivity at any time. In contrast, the 3-O-methylated products represented a significant percentage of the total radioactivity: [^{18}F]3-OMFD (40–50%) and [^{3}H]3-OMD (20–25%).

In the cerebellum, the radioactivity was identified as that of [^{18}F]FDOPA, [^{18}F]3-OMFD, [^{3}H]DOPA and [^{3}H]3-OMD (Fig. 6). From 15 to 60 min the percent of [^{18}F]3-OMFD ranged from 70 to 90%, whereas that of [^{3}H]3-OMD ranged from 30 to 52% of the total radioactivity. [^{18}F]FDOPA levels remained lower relative to those of [^{3}H]DOPA; the percent of [^{18}F]FDOPA declined from 28 to 5%, whereas that of [^{3}H]DOPA declined from 60 to 35% between 15 and 60 min. [^{18}F]FDA sulfate and [^{18}F]FHVA activities were less than 5% throughout the experiment. [^{3}H]HVA and [^{3}H]DA sulfate activity did not exceed 10%.

The volumes of distribution for [^{18}F]3-OMFD and [^{3}H]3-OMD in the striatum and cerebellum were equivalent, indicated by the [^{18}F]3-OMFD or [^{3}H]3-OMD striatum (counts/g)/cerebellum (counts/g) ratio of approximately one for each isotope from 30 to 60 min (Fig. 7).

DISCUSSION

The validity of the use of [^{18}F]FDOPA as a biochemical probe of presynaptic dopaminergic function with PET is predicated on the assumption that [^{18}F]FDOPA in the central nervous system follows the metabolic pathway of endogenous dopamine.

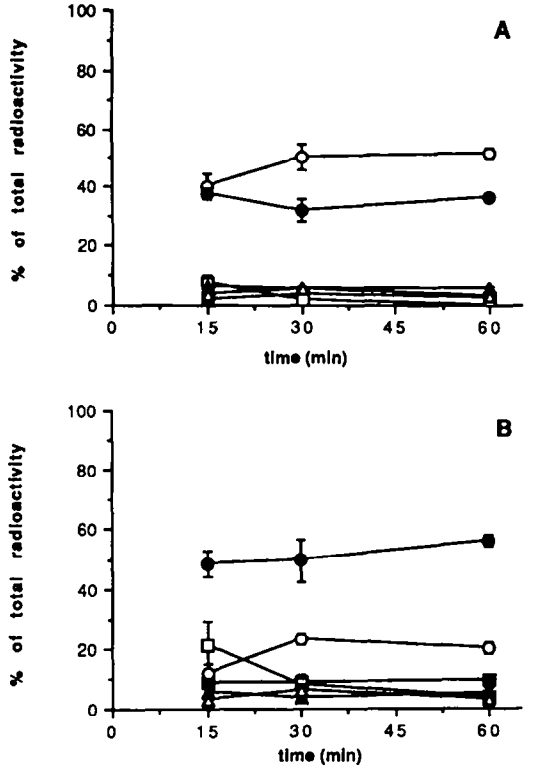

Fig. 5. Striatum metabolite profiles expressed as percent of total radioactivity. Animals were pretreated with carbidopa (5 mg/kg, s.c.) 60 min before injection (10–20 sec) of [18F]FDOPA [800 μCi/kg (0.4 to 0.8 μmol/kg) i.v.] and [3H]DOPA [150 μCi/kg (3.8 nmol/kg) i.v.]. Key: Panel A: [18F]3-OMFD (○), [18F]FDA (●), [18F]FDOPAC (▲), [18F]FDOPA (□), [18F]FHVA (△), and [18F]FDA sulfate (■); Panel B: [3H]DA (●), [3H]3-OMD (○), [3H]DOPA (□), [3H]DA sulfate (■), [3H]DOPAC (▲), and [3H]HVA (△). Each data point is the mean ± SD from values of three to five animals.

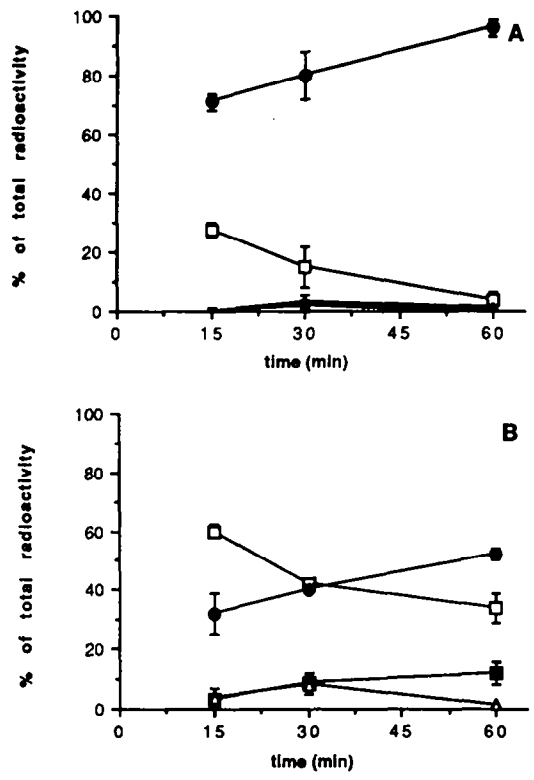

Fig. 6. Cerebellum metabolite profiles expressed as percent of total radioactivity. Animals were pretreated with carbidopa (5 mg/kg, s.c.) 60 min before injection (10–20 sec) of [18F]FDOPA [800 μCi/kg (0.4 to 0.8 μmol/kg) i.v.] and [3H]DOPA [150 μCi/kg (3.8 nmol/kg) i.v.]. Key: Panel A: [18F]3-OMFD (●), [18F]FDOPA (□), [18F]FDA sulfate (■), and [18F]FHVA (△); Panel B: [3H]DOPA (□), [3H]3-OMD (●), [3H]HVA (△), and [3H]DA sulfate (■). Each data point is the mean ± SD from values of three to five animals.

Striatum to cerebellum total activity ratios have been used in [18F]FDOPA studies with PET to differentiate specific striatal monoaminergic processes from non-specific brain metabolism and transport of metabolites from peripheral tissues. We have found that in rats [3H]DOPA and [18F]FDOPA show parallel increases in the striatum to cerebellum total activity ratios (Fig. 3), from which specific biochemical mechanisms of striatal function can be inferred as responsible for this accumulation. The decarboxylation of [18F]FDOPA and [3H]DOPA in brain tissue is indicated by the formation of [18F]FDA and [3H]DA respectively. The aromatic amino acid decarboxylase (AAAD) mediated transformation is specific for striatal tissue since no radioactive monoamines were observed in cerebellar tissue, which lacks dopamine terminals [11]. It has been shown earlier in rat corpus striatum that conversion of exogenous L-DOPA to DA [12], and [3H]DOPA to [3H]DA and its metabolites [3H]DOPAC and [3H]HVA [13] occurs mainly in dopaminergic neurons. None of the striatal AAAD contained within serotonergic neurons [14], nor-

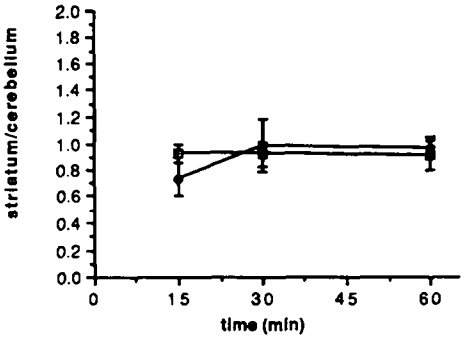

Fig. 7. Striatum (counts/g)/cerebellum (counts/g) ratios for [18F]3-OMFD (■) and [3H]3-OMD (○). Each data point is the mean ± SD from values of three to five animals.

adrenergic neurons [15] or cerebral capillaries [16] appears to contribute significantly to DA formation after exogenous L-DOPA administration [12].

The rapid *in vivo* decarboxylation of both [18F]FDOPA and [3H]DOPA in striatal dopaminergic terminals can also be expected from existing

in vitro data. The K_m determined for 6-fluoro-L-DOPA for AAAD (100 μM) [17] is well within the range of that determined for L-DOPA (40–200 μM) [18, 19]. On the other hand, the low levels of further products of central oxidation of monoamines, namely [^{3}H]DOPAC and [^{3}H]HVA for [^{3}H]DOPA, and [^{18}F]FDOPAC and [^{18}F]FHVA for [^{18}F]FDOPA, indicate that both [^{3}H]DOPA and [^{18}F]FDOPA are not metabolized extensively at 15–60 min post injection beyond conversion to [^{3}H]DA and [^{18}F]FDA respectively. This is also indicated from the minimal decrease in the absolute levels of [^{18}F]FDA (20%) and [^{3}H]DA (25%) throughout this same time period. These results therefore suggest that [^{3}H]DA and [^{18}F]FDA derived from exogenously administered [^{3}H]DOPA and [^{18}F]FDOPA respectively, are mainly stored in a slow turnover rate functional pool. Earlier work has demonstrated the existence of multiple DA pools in striatal neurons [20]. There are data to indicate that the releasable DA pool is smaller and more dynamic (turnover rate = 2.5 hr^{-1}) than the larger DA pool that has a slow turnover rate (k = 0.21 hr^{-1}) [21, 22].

Interestingly, [^{18}F]FDA essentially mimics central [^{3}H]DA metabolism, which confirms the notion that [^{18}F]FDOPA is a functional analog of exogenous L-DOPA for evaluation of central dopaminergic mechanisms. Evidence for a similar functional storage pool for [^{18}F]FDA and DA was also provided by Chieuh *et al.* [23], who found that [^{18}F]FDA accumulation could be depleted by reserpine, and also released by elevated (60 mM) K^+ concentrations. However, our data provide no evidence that a relationship between [^{18}F]FDA synthesis from exogenous [^{18}F]FDOPA and DA synthesis from endogenous tyrosine exists.

The 3-O-methylated products are *in vivo* metabolites for both [^{18}F]FDOPA ([^{18}F]3-OMFD) and [^{3}H]DOPA ([^{3}H]3-OMD). Both [^{18}F]3-OMFD and [^{3}H]3-OMD could be identified as major metabolites in plasma and brain tissue. For example, at 60 min [^{18}F]3-OMFD accounts approximately for 80 and 90% of the total activity in plasma and cerebellum respectively. In striatal tissue, [^{18}F]3-OMFD remained the major metabolite identified throughout the experimental period. However, the nonvariant striatum/cerebellum distribution for [^{18}F]3-OMFD, indicated by its uniform distribution in both areas of the brain, suggests a peripheral origin of the [^{18}F]3-OMFD. This allows for accurate predictions of its concentration in striatum and cerebellum based on plasma concentrations, a crucial factor in the development of accurate tracer kinetic models with PET [24].

From the available data (Figs. 2B, 5A and 6A), it becomes clearly evident that the peripheral formation of [^{18}F]3-OMFD from [^{18}F]FDOPA exceeds that of [^{3}H]3-OMD from [^{3}H]DOPA at similar times. This may account, in part, for a more rapid decrease of plasma levels of [^{18}F]FDOPA relative to that of [^{3}H]DOPA. The rapid and significant 3-O-methylation of [^{18}F]FDOPA follows known biochemical mechanisms of *in vivo* methylation of catechols. The central enzyme to this pathway is catechol-*O*-methyltransferase (COMT, EC 2.1.1.6) that catalyzes the transfer of the methyl group from *S*-adenosylmethionine to one of the phenolic groups of the catechol substrate by a random Bi Bi mechanism [25]. Formation of a S_N2-like transition state [26] is central to the catalytic process for which it was postulated that changes in the acidity of the phenolic hydroxyl groups would produce (a) modification in the apparent affinity with the enzyme, and (b) changes in the regioselectivity in the COMT-catalyzed O-methylation [27, 28]. The effect of fluorine substitution on the kinetics of catechol substrates for COMT was studied extensively by Kirk and Creveling [29], who noted an apparent correspondence between substrate phenolic pK_a and enzyme affinity (e.g. K_m), with the most acidic substrates having the highest enzyme affinities. Since fluoro-L-DOPA analogs are expected to have the same acidity patterns for their phenolic groups as fluorodopamine or fluoronorepinephrine (2-F = 5-F > 6-F > H) [27], one would expect, assuming no other intervening factors, that [^{18}F]FDOPA would be a similar or better substrate for COMT relative to L-DOPA. This hypothesis was confirmed by our *in vivo* results.

The increase in 3-O-methylation of 6-fluoro-L-DOPA relative to L-DOPA as observed in the present work could be rationalized as due to the perturbations of the electron density caused by the fluorine substitution on the aromatic ring. STO-3G level *ab initio* molecular orbital calculations of a number of substituted phenols clearly indicate that in ortho- and parafluorophenols, the fluorine atom destabilizes the phenolic "OH" group and stabilizes the phenoxide ion [30]. Also in meta-fluorophenol, the fluorine atom tends to destabilize the phenoxide and stabilize the phenolic "OH" group. These interactions are attributed to the σ-accepting and π-donating inductive effect of the fluorine substitution. Analogue theoretical calculations on substituted fluorocatechols also endorse such σ- and π-interactions and stabilizations of catechoxide/catechol [31]. An excellent linear correlation observed between the gas-phase and aqueous acidity of fluoro- and other substituted phenols [32] makes the above rationale valid for the present discussion.

A destabilization of one of the phenolic groups to the corresponding phenoxide in any catechol would increase its nucleophilicity leading to its selective methylation by COMT. Thus, at physiological pH, in 6-fluoro-L-DOPA the 4-OH group (which is meta with reference to the fluorine substitution) would be stabilized as the phenolic "OH" group, whereas the 3-OH group (which is para to the fluorine substitution) would be stabilized as its phenoxide ion. This results in a preferential and increased 3-O-methylation by COMT.

It has been reported recently, however, that the rate of O-methylation of fluorinated DOPA analogs using COMT purified from rat liver follows the sequence 2-F $\cong$ 5-F > H $\gg$ 6-F [33]. The reversal in *in vitro* affinity with COMT for 6-fluoro-DOPA and L-DOPA appears to contradict earlier observations with substituted fluorodopamines and fluoroepinephrines [27] and is not confirmed by data presented in this work. It should be pointed out, however, that the *in vitro* results [33] have to be interpreted with caution, because experiments were

conducted with racemic mixtures of 6-fluoroDOPA at pH 9.0. In addition to a drastic departure from physiological conditions, the use of the racemate may have produced results different from those obtained by the use of the L-isomer insofar as recent evidence indicates stereoselectivity for COMT [34].

In summary, the results of this work show that the peripheral metabolism of [^{18}F]FDOPA resembles that of L-DOPA with rapid formation of [^{18}F]3-OMFD. This observation is in disagreement with earlier work *in vitro* [33] and *in vivo* [4], but is in agreement with known mechanisms of methyl group transfer catalyzed by COMT [25]. The validity of these observations is not dependent on mechanisms of AAAD regulation of [^{18}F]FDOPA availability, nor is it restricted to the animal species used. For example, significant O-methylation of 6-fluoro-L-DOPA was observed earlier in the presence or absence of carbidopa [35], an AAAD inhibitor used with L-DOPA in the treatment of Parkinson's disease, and in studies with hooded rats [3]. Moreover, [^{18}F]3-OMFD was found to be the major peripheral metabolite of [^{18}F]FDOPA in our studies with non-human primates (*Macaca nemestrina* monkeys) and humans [36]. The peripheral metabolism of [^{18}F]FDOPA observed in those studies parallels the results obtained in this work with rats and thus substantiates the use of this animal species as a model for [^{18}F]FDOPA metabolism in humans. A full account of these observations will be published elsewhere.

The comparative *in vivo* metabolism of [^{3}H]DOPA and [^{18}F]FDOPA presented in this work provides the basis for the development of quantitative tracer kinetic models of striatal dopaminergic function with PET. For example, these rat data have already been successfully applied to the formulation of kinetic models of FDOPA metabolism in humans [37]. We have presented data to indicate that the peripheral and cerebral metabolism of [^{18}F]FDOPA does qualitatively reflect that of exogenously administered L-DOPA. Further work is now required to determine whether any quantitative correlation can be established between [^{18}F]FDOPA kinetic behavior and the *in vivo* synthesis of labeled dopamine from exogenously labeled tyrosine, modulated by the rate-limiting enzyme, tyrosine hydroxylase [38].

Acknowledgements—We would like to thank our colleague Dr. N. Satyamurthy for molecular orbital analysis of a number of substituted phenols. This work was supported in part by Contract DE-AC03-76-SF00012 from the Department of Energy, Grants R01-NS-20867 and P01-NS-15654 from the National Institutes of Health, Grant R01-MH-37916 from the National Institute of Mental Health, and donations from the Hereditary Disease Foundation and the Jennifer Jones Simon and Ahmanson Foundation.

REFERENCES

1. Mazziotta JC and Phelps ME, Positron emission tomography studies of the brain. In: *Positron Emission Tomography and Autoradiography* (Eds. Phelps ME, Mazziotta JC and Schelbert HR), pp. 493–580. Raven Press, New York, 1986.
2. Barrio JR, Huang S-C and Phelps ME, *In vivo* assessment of neurotransmitter biochemistry in humans. *Annu Rev Pharmacol Toxicol* **28**: 213–230, 1988.
3. Cumming P, Boyes BE, Martin WRW and Adam M, The metabolism of [^{18}F]6-fluoro-L-3,4-dihydroxyphenylalanine in the hooded rat. *J Neurochem* **48**: 601–608, 1987.
4. Firnau G, Sood S, Chirakal R, Nahmias C and Garnett ES, Cerebral metabolism of 6-[^{18}F]fluoro-L-3,4-dihydroxyphenylalanine in the primate. *J Neurochem* **48**: 1077–1082, 1987.
5. Belbeck L, Sood S, Nahmias C, Firnau G and Garnett ES, Blood–brain barrier transport and cerebral utilization of dopa in living monkeys. *Am J Physiol* **238** (Regulatory Integrative Comp. Physiol. 7): R318–R327, 1980.
6. Firnau G, Sood S, Chirakal R, Nahmias C and Garnett ES, Metabolites of 6-[^{18}F]fluoro-L-DOPA in human blood. *J Nucl Med* **29**: 363–369, 1988.
7. Luxen A and Barrio JR, Fluorination of substituted veratroles via regioselective mercuration. *Tetrahedron Lett* **29**: 1501–1504, 1988.
8. Luxen A, Bida GT, Phelps ME and Barrio JR, Syntheses of enantiomerically pure D and L 6-[F-18]fluorodopa and *in-vivo* metabolites via regioselective fluorodemercuration. *J Nucl Med* **28**: 624, 1987.
9. Shoaf SE and Elchisak MA, Distribution of free and conjugated dopamine in rats and mice. *Life Sci* **33**: 625–630, 1983.
10. Johnson GA, Baker CA and Smith RT, Radioenzymatic assay of sulfate conjugates of catecholamines and dopa in plasma. *Life Sci* **26**: 1591–1598, 1980.
11. Murrin LC and Kuhar MJ, Dopamine receptors in the rat frontal cortex: An autoradiographic study. *Brain Res* **177**: 279–285, 1979.
12. Hefti F, Melamed E and Wurtman RJ, The site of dopamine formation in rat striatum after L-DOPA administration. *J Pharmacol Exp Ther* **217**: 189–197, 1981.
13. Horne MK, Cheng CH and Wooten GF, The cerebral metabolism of L-dihydroxyphenylalanine. *Pharmacology* **28**: 12–26, 1984.
14. Melamen E, Hefti F and Wurtman RJ, L-3,4-Dihydroxyphenylalanine and L-5-hydroxytryptophan decarboxylase activities in rat striatum: Effects of selective destruction of dopaminergic or serotonergic input. *J Neurochem* **34**: 1753–1756, 1980.
15. Hökfelt T, Fuxe K and Goldstein M, Immunohistochemical localization of aromatic L-amino acid decarboxylase (DOPA decarboxylase) in central dopamine and 5-hydroxytryptamine nerve cell bodies of the rat. *Brain Res* **53**: 175–180, 1973.
16. Butler A, Falck B, Owman C and Rosengren E, The localization of monoaminergic blood–brain barrier mechanisms. *Pharmacol Rev* **18**: 369–385, 1966.
17. Cumming P, Hauser M, Martin WRW, Grierson J, Adam MJ, Ruth TJ and McGeer EG, Kinetics of *in vitro* decarboxylation and the *in vivo* metabolism of 2-^{18}F- and 6-^{18}F-fluorodopa in the hooded rat. *Biochem Pharmacol* **37**: 247–250, 1988.
18. Okuno S and Fujisawa H, Accurate assay of dopa decarboxylase by preventing nonenzymatic decarboxylation of dopa. *Anal Biochem* **129**: 412–415, 1983.
19. Bosin TR, Baldwin JR and Maickel RP, Inhibition of DOPA decarboxylation by analogues of tryptophan. *Biochem Pharmacol* **27**: 1289–1291, 1978.
20. Gropetti A, Algeri S, Cattaheni F, DiGiulio AM, Galli GL, Ponzio F and Spano PF, Changes in specific activity of dopamine metabolites as evidence of a multiple compartmentation of dopamine in striatal neurons. *J Neurochem* **28**: 193–197, 1977.

21. Michael AC, Justice JB Jr and Neill DB, *In vivo* voltammetric determination of the kinetics of dopamine metabolism in the rat. *Neurosci Lett* **56**: 365–369, 1985.
22. Wood PL, Kim HS, Stocklin K and Rao TS, Dynamics of the striatal 3-MT pool in rat and mouse: Species differences as assessed by steady-state measurements and intracerebral dialysis. *Life Sci* **42**: 2275–2281, 1988.
23. Chieuh CC, Zukowska-Grojec Z, Kirk KL and Kopin IJ, 6-Fluorocatecholamines as false adrenergic neurotransmitters. *J Pharmacol Exp Ther* **225**: 529–533, 1983.
24. Huang S-C, Carson RE and Phelps ME, Tracer kinetic modeling in positron computer tomography. In: *Tracer Kinetics and Physiological Modeling* (Eds. Lambrecht RM and Rescigno A), pp. 298–344. Springer, New York, 1983.
25. Woodward RW, Tsai M-D, Floss HG, Crooks PA and Coward JK, Stereochemical course of the transmethylation catalyzed by catechol-*O*-methyltransferase. *J Biol Chem* **255**: 9124–9127, 1980.
26. Hegazi MF, Borchardt RT and Schowen RL, α-Deuterium and carbon-13 isotope effects for methyl transfer catalyzed by catechol-*O*-methyltransferase. S_N2-like transition state. *J Am Soc* **1011**: 4359–5365, 1979.
27. Kirk KL, Cantacuzene D, Nimitkitpaisan Y, McCulloh D, Padgett WL, Daly JW and Creveling CR, Synthesis and biological properties of 2-,5-,6-fluoronorepinephrines. *J Med Chem* **22**: 1463–1467, 1979.
28. Creveling CR, McNeal ET, Cantacuzene D and Kirk KL, Influence of the fluorine substitutions on the site of enzymatic O-methylation of fluorinated norepinephrines. *J Med Chem* **24**: 1395–1399, 1981.
29. Kirk KL and Creveling CR, The chemistry and biology of ring-fluorinated biogenic amines. *Med Res Rev* **4**: 189–220, 1984.
30. Pross A, Radom L and Taft RW, Theoretical approach to substituent effects. Phenols and phenoxide ions. *J Org Chem* **45**: 818–826, 1980.
31. Hodoscek M and Solmajer T, Effects of fluorine substitution in the catechole ring. An *ab-initio* MO theoretical study. *Theor Chim Acta (Berl)* **66**: 395–403, 1985.
32. McMahon TB and Kebarle P, Intrinsic acidities of substituted phenols and benzoic acids determined by gas-phase proton-transfer equilibria. *J Am Chem Soc* **99**: 2222–2230, 1977.
33. Creveling CR and Kirk KL, The effect of ring-fluorination on the rate of O-methylation of dihydroxyphenylalanine (DOPA) by catechol-*O*-methyltransferase: Significance in the development of ^{18}F-PETT scanning agents. *Biochem Biophys Res Commun* **130**: 1123–1131, 1985.
34. Gordonsmith RH, Raxworthy MJ and Gulliver PA, Substrate stereospecificity and selectivity of catechol-*O*-methyltransferase for DOPA, DOPA derivatives and α-substituted catecholamines. *Biochem Pharmacol* **31**: 433–437, 1982.
35. Melega WP, Luxen A, Perlmutter M, Phelps ME and Barrio JR, Effects of carbidopa on the peripheral and cerebral metabolism of 6-[^{18}F]-L-fluorodopa in rodents. *Pharmacologist* **30**: A54, 1988.
36. Melega WP, Hoffman JM, Luxen A, Perlmutter M, Huang S-C, Hawk TC, Grafton S, Mahoney K, Mazziotta JC, Phelps ME and Barrio JR, Effects of carbidopa on the peripheral and cerebral 6-[^{18}F]fluoro-L-DOPA metabolism in monkeys and humans: Tracer kinetic correlates for positron emission tomography. *J Cereb Blood Flow Metab* **9**: (Suppl 1): S420, 1989.
37. Huang SC, Barrio JR, Hoffman JM, Mahoney DK, Hawk TC, Melega WP, Luxen A, Perlmutter M, Grafton S, Mazziotta JC and Phelps ME, A compartmental model for 6-[F-18]fluoro-L-DOPA kinetics in cerebral tissues. *J Nucl Med* **30**: 735, 1989.
38. Maserano JM and Weiner N, Tyrosine hydroxylase regulation in the central nervous system. *Mol Cell Biochem* **53/54**: 129–152, 1983.