Metabolites of 6-[^{18}F]Fluoro-L-Dopa in Human Blood

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The metabolites of 6-[^{18}F]fluoro-L-dopa in the blood plasma of healthy humans have been identified as 3-O-sulfato-6[^{18}F]fluoro-L-dopa, 3-O-methyl-6-[^{18}F]fluoro-L-dopa, 6-[^{18}F] fluorodopamine, and 6-[^{18}F]fluorohomovanillic acid. The time course of these metabolites was followed up to 2 hr. The findings have implications for the use of 6-[^{18}F]fluoro-L-dopa as tracer for cerebral dopamine metabolism. Despite the variety of metabolites in the peripheral blood there are only two ^{18}F-carrying compounds, 6-[^{18}F]fluoro-L-dopa and 3-O-methyl-6-[^{18}F]fluoro-L-dopa, that can cross the blood-brain barrier. After 1 hr, the plasma concentration of 3-O-methyl-6-[^{18}F]fluoro-L-dopa reaches ~20% that of 6-[^{18}F]fluoro-L-dopa but the mean concentration of the O-methylated metabolite over the same interval is <5% that of 6-[^{18}F]-fluoro-L-dopa.

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Positron tomography (PT) used with the tracer 6-[^{18}F] fluoro-L-dopa is able to visualize the dopaminergic pathways in the human brain during life (*1*). In rhesus monkeys, the retention of fluorine-18 (^{18}F) in the cerebral dopaminergic regions (such as striatum) is predominantly due to 6-[^{18}F]fluoro-dopamine which was derived in vivo from 6-[^{18}F]fluoro-L-dopa (*2*). The similarity of the neurochemistry of catecholamines among primates may permit us to extend this result to the human brain. The biochemical lesion in the brains of patients with Parkinson's disease has been demonstrated by this method. In the striatum of these patients the neurotransmitter dopamine is reduced and, concomitantly, the striata accumulate less ^{18}F than normal (*3–7*).

The PT method with 6-[^{18}F]fluoro-L-dopa has the potential to measure quantitatively the kinetics of cerebral dopamine metabolism. Quantitative analysis of PT data requires a mathematic model which describes the metabolic fate and the time course of the tracer 6-[^{18}F]fluoro-L-dopa in the brain as well as in the blood. The metabolites in the brain of primates are known (*2*), but those in the human blood are not.

In this paper we report on the metabolic fate of 6-[^{18}F]fluoro-L-dopa in the blood of humans and compare our results with those obtained with L-[^{14}C]dopa. This knowledge will facilitate the development of a quantitative method for intracerebral dopamine metabolism.

The metabolism of the parent molecule L-dopa in the human blood has been summarized recently (*8*). L-Dopa is transformed predominantly by aromatic amino acid decarboxylase (dopa decarboxylase) to dopamine and, to a smaller extent, by catechol-O-methyl-transferase to 3-O-methyl-L-dopa (*9*). L-Dopa can also be O-sulfonated (*10*) and participates in transamination (Fig. 1). After administration of 6-[^{18}F]fluoro-L-dopa a similar pattern of ^{18}F carrying metabolites can be expected.

MATERIALS AND METHODS

6-[^{18}F]Fluoro-L-dopa was synthesized as already described (*11,12*). The expected metabolites 6-[^{18}F]fluoro-dopamine, 3-O-methyl-6-[^{18}F]fluoro-L-dopa, 6-[^{18}F]fluoro-3,4-dihydroxyphenyl acetic acid and 6-[^{18}F]fluoro-homovanillic acid were synthesized by the general fluorination technique for catechol compounds (*11*). These compounds were characterized by their molecular ion in high resolution mass spectroscopy with fast atom bombardment ionization and their ^{19}F-nuclear magnetic resonance (NMR) spectra (*13*). They served as authentic materials to calibrate the extraction procedure and the chromatographic analysis of the plasma extracts. Comparisons were made with L-[^{14}C]dopa and [7-^{14}C]dopamine. Both carbon-14- (^{14}C) labeled agents were obtained from the Radiochemical Centre, Amersham. Blood metabolites were measured in nine volunteers as part of a 6-[^{18}F]fluoro-L-dopa/PT scan. The age of the volunteers ranged from 29 to 65 yr.

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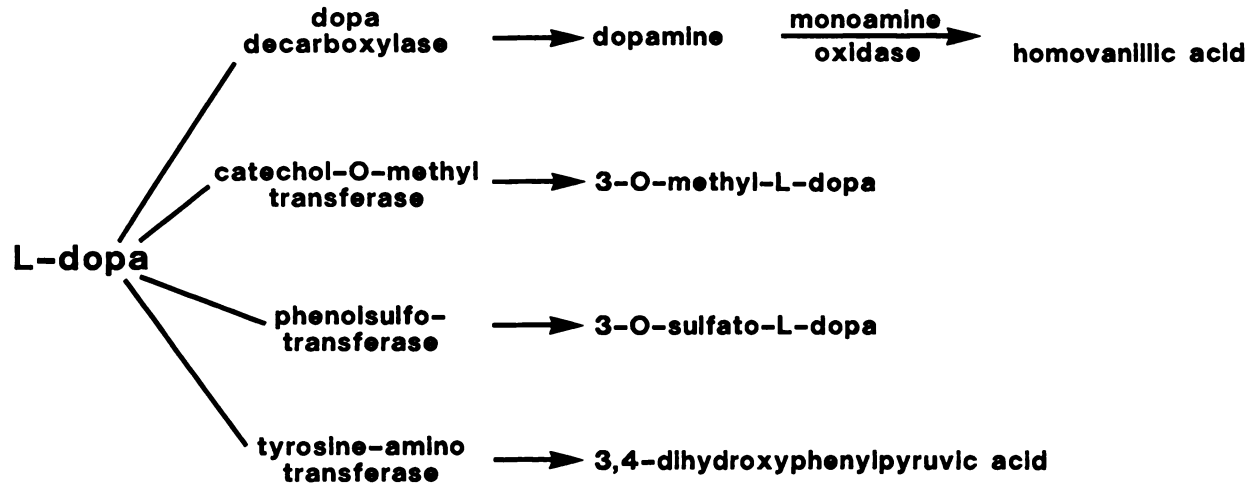

FIGURE 1
Metabolism of L-dopa in the periphery.

Analysis of ^{18}F Metabolites

6-[^{18}F]Fluoro-L-dopa (4 to 6 mCi, specific activity 140–170 mCi/mmol) was injected intravenously. Arterial or venous blood samples (in each of two subjects) were taken at intervals after the tracer had been injected. Fluorine-18 was measured by gamma scintillation counting. Chemical analyses were made on plasma obtained by centrifugation at 2,000 *g* for 5 min. The plasma proteins were precipitated with 7% perchloric acid and separated by centrifugation at 2,000 *g* for 10 min. The supernatant was concentrated to 500 μl by rotary evaporation and then passed through a 0.22-μm membrane filter. Fluorine-18-containing metabolites were separated by liquid chromatography (column: Regis-Hichrom, 5μ, 4.6 × 250 mm; mobile phase: 10% methanolic solution of sodium dihydrogenphosphate (75 m*M*) buffer adjusted to pH 2.9 with 70% perchloric acid, containing 1 μ*M* disodium EDTA and 1 m*M* sodium octyl sulfate; flowrate 1 ml/min). The effluent from the column was collected in 2 ml fractions by an automatic fraction collector. The ^{18}F in each fraction was measured with a NaI(Tl) gamma scintillation counter. The ^{18}F-containing peaks were identified by comparing their elution times with those of the authentic ^{18}F-labeled catechol materials which were chromatographed under identical conditions. The total ^{18}F content of the plasma extract was never < 10,000 cpm, even at later time points.

The losses that each metabolite suffered during the extraction procedure and hence the extraction efficiency were determined in separate experiments. Authentic 6-[^{18}F]fluoro-L-dopa, 6-[^{18}F]fluoro-L-dopamine, 3-O-methyl-6-[^{18}F]fluoro-L-dopa or 6-[^{18}F]fluorohomovanillic acid (~5.0 μCi each) were added to 1 ml of human plasma and the mixture was processed as outlined above. The fraction of the added ^{18}F that was recovered after the extraction procedure and chromatographic analysis was 0.623 for 6-[^{18}F]fluoro-L-dopa, 0.625 for 6-[^{18}F] fluoro-L-dopamine, 0.628 for 3-O-methyl-6-[^{18}F]fluoro-L-dopa and 0.450 for 6-[^{18}F]fluorohomovanillic acid. The ^{18}F in each chromatographic analysis was corrected for half-life ($T_{1/2}$ of ^{18}F is 110 min) and the ^{18}F in each metabolite peak was then corrected for its corresponding recovery. Fluorine-18 associated with each metabolite was finally expressed as percent of the total ^{18}F contained in the plasma.

An unexpected peak fraction No. 4 occurred in the high performance liquid chromatography (HPLC) analysis of the plasma. This peak was tentatively assigned as 6-[^{18}F]fluoro-O-sulfato-L-dopa for the following reasons.

First, when the fraction was hydrolyzed with 0.3*M* HCl at 100° for 40 min (*14*) and re-chromatographed and it was found that the majority of the ^{18}F eluted in the 6-[^{18}F]fluoro-L-dopa fractions (Fig. 1). Second, when L-dopa was converted to 3- and 4-O-sulfato-L-dopa by the reaction with concentrated sulfuric acid at 0°C for 1 hr (*15*) and an aliquot of this reaction mixture was chromatographed (chromatographic conditions as above; eluate monitored with uv detector at 280 nm) the amount of L-dopa was drastically reduced and two new not quite resolved peaks appeared in the position of fractions Nos. 3 and 4. This indicates the formation of 3- and 4-O-sulfato-L-dopa that are known to elute separately and well ahead of L-dopa on reversed phase liquid chromatographic columns (*16*).

Analysis of ^{14}C Metabolites

In one individual, L-[^{14}C]dopa (50 μCi, specific activity 10.9 mCi/mmol) and 6-[^{18}F]fluoro-L-dopa were co-injected and venous blood samples were collected at intervals after the injection. Each blood sample was treated as described above. The chromatographic analysis of the ^{14}C-metabolites was done differently from that used with 6-[^{18}F]fluoro-L-dopa to optimize separation between ^{14}C-metabolites (column: Waters μ-Bondapak C-18, 10 μ, 7.8 × 300 mm; mobile phase: 4% methanolic solution of sodium dihydrogenphosphate buffer pH 2.9 with 1 m*M* sodium octylsulfate and 1 μ*M* disodium EDTA, flow rate 2 ml/min; uv-detector at 280 μm). Authentic, nonradioactive, L-dopa, 3-O methyl-L-dopa, dopamine and homovanillic acid (Aldrich Chemical Company, Milwaukee, WI) were added to the sample before HPLC analysis to mark the elution positions and thus identify the ^{14}C-peaks. Eluate was collected in 2 ml fractions. Each fraction was transferred into a scintillation vial and the liquid was evaporated at room temperature in a stream of nitrogen. Scintillation cocktail (Biofluor, New England Nuclear, 15 ml) was added to each vial. After the ^{18}F had decayed the ^{14}C content of each fraction was measured in an LKB 1219 Rackbeta scintillation counter using the internal standard method. Recovery was determined

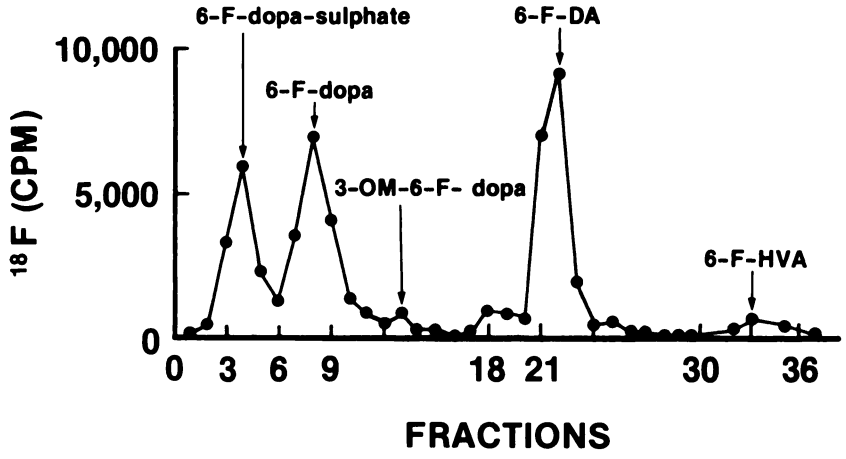

FIGURE 2
Liquid chromatogramme of the extract of human blood plasma taken 30 min after injection of 6-[^{18}F]fluoro-L-dopa.

as described for the ^{18}F-metabolites. Using L-[^{14}C]dopa and [7-^{14}C]dopamine the fraction of ^{14}C recovered after the extraction procedure and chromatographic analysis was 0.620 and 0.542, respectively. O-Methyl-L-[^{14}C]dopa and [^{14}C]homovanillic acid were not commercially available so their recoveries were assumed to be the same as those of the fluorinated analogs. The ^{14}C in each metabolite fraction was corrected for its corresponding recovery. The ^{14}C associated with each metabolite was then expressed as percent of the total ^{14}C contained in the plasma.

Reaction in vitro between fluoro-dopas and S-adenosylmethionine catalyzed by catechol-O-methyltranferase. The reaction mixture with 6-[^{18}F]fluoro-L-dopa as substrate was prepared according to the general method by Coward and Wu (*17*). The incubation time was 2 hr. The analysis of the reaction mixture was described in detail earlier (*18*).

The relation between 3-O-methyl-6-[^{18}F]fluoro-l-dopa in the plasma and ^{18}F in the brain. One of the nine subjects who was given 6-[^{18}F]fluoro-L-dopa was given, on a separate occasion, an i.v. dose of 3-O-methyl-6[^{18}F]fluoro-L-dopa (3 mCi). Arterial blood samples were collected for 2 hr during which time the accumulation and distribution of ^{18}F in the brain was measured in the McMaster Positron Tomograph (*19*). Tomographic slices (1.6 cm thick) which contained striatum and occipital cortex were examined.

RESULTS

The 1.8 hr half-life of ^{18}F required a chromatographic procedure that separated the basic and acidic metabolites in a single analysis. Figure 2 shows a typical chromatogram: it demonstrates a good separation between the metabolites.

Five major ^{18}F-containing metabolites were found and identified: 6-[^{18}F]fluoro-O-sulfato-L-dopa, 6-[^{18}F] fluoro-L-dopa, 3-O-methyl-6-[^{18}F]fluoro-L-dopa, 6-[^{18}F] fluoro-dopamine and 6-[^{18}F]fluoro-homovanillic acid.

In two individuals the time course of ^{18}F in arterial and venous whole blood was measured. The data from one of these individuals is shown in Figure 3 which is representative of both studies. During the first 15 min after the injection of 6-[^{18}F]fluoro-L-dopa arterial and venous disappearance curves have different shapes.

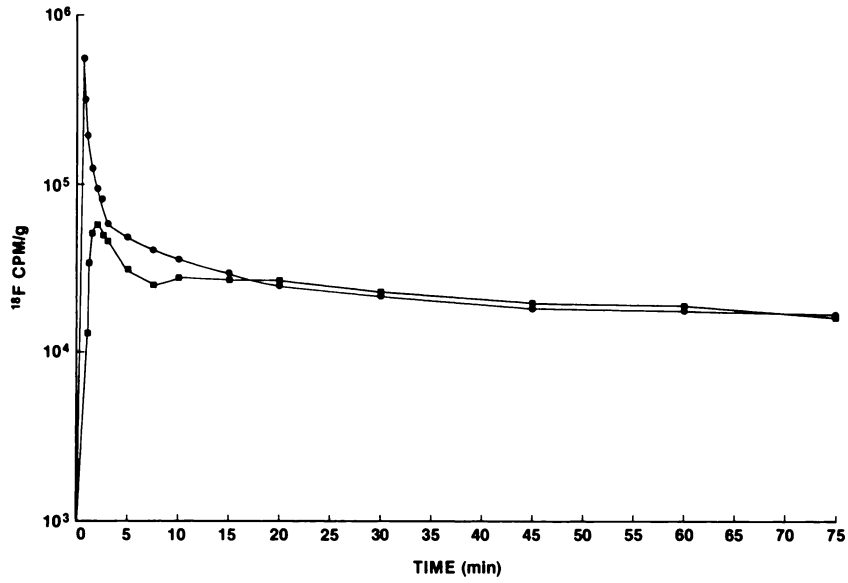

FIGURE 3
Clearance of ^{18}F from human arterial (●) and from venous (■) blood after an injection of 6-[^{18}F]fluoro-L-dopa at zero time

The disappearance of ^{14}C and ^{18}F from arterial blood plasma after the simultaneous injection of L-[^{14}C]dopa and 6-[^{18}F]fluoro-L-dopa was measured in one individual and is shown in Figure 4A. Fluorine-18 disappears faster than ^{14}C.

The results of the chromatographic analyses at various times, both in arterial and in venous blood plasma are shown in Figures 4B, 4C and 4D. Figure 4B shows the pooled results from nine individuals, each studied at six time points. 6-[^{18}F]Fluoro-homovanillic acid was not sought in all samples. The major metabolites are 6-[^{18}F]fluoro-dopamine and 3-O-sulfato-6-[^{18}F]fluoro-L-dopa.

Figures 4C and 4D show the analysis of venous plasma from the same individual after co-injection of L-[^{14}C]dopa and 6-[^{18}F]fluoro-L-dopa. 6-[^{18}F]Fluoro-dopa leaves the plasma faster than dopa and more fluoro-dopamine and 3-O-sulfato-fluoro-L-dopa are made. In contrast L-[^{14}C]Dopa is mainly O-methylated. This observation was confirmed both in vivo (Fig. 5) and in vitro, (Table 1). L-Dopa appears to be methylated four times faster than 6-fluoro-L-dopa.

Figure 6 shows, on a log scale, the amount of ^{18}F, with time after injection, that is contained in one ml of whole blood, the amount of ^{18}F that is contained in the plasma from 1 ml of whole blood, the amount of ^{18}F associated with fluoro-L-dopa and the amount of ^{18}F associated with 3-O-methyl-6-fluoro-L-dopa that is contained in 1 ml of whole blood. Data from nine individuals are pooled.

When 3-O-methyl-6-[^{18}F]fluoro-L-dopa was injected ^{18}F activity was uniformly distributed throughout the gray structures of the brain. At 1 hr the concentration of ^{18}F in the striatum was the same as that in the occipital cortex (110 cpm per pixel per mCi injected). The time integral of the arterial plasma ^{18}F active activity over the first hour was 775,000 cpm·hr·mCi^{-1}. This compared with a time integral of ^{18}F activity from 3-O-methyl-6-[^{18}F]fluoro-L-dopa derived from 6-[^{18}F] fluoro-L-dopa of 14,800 cpm·hr·mCi^{-1}. in the same

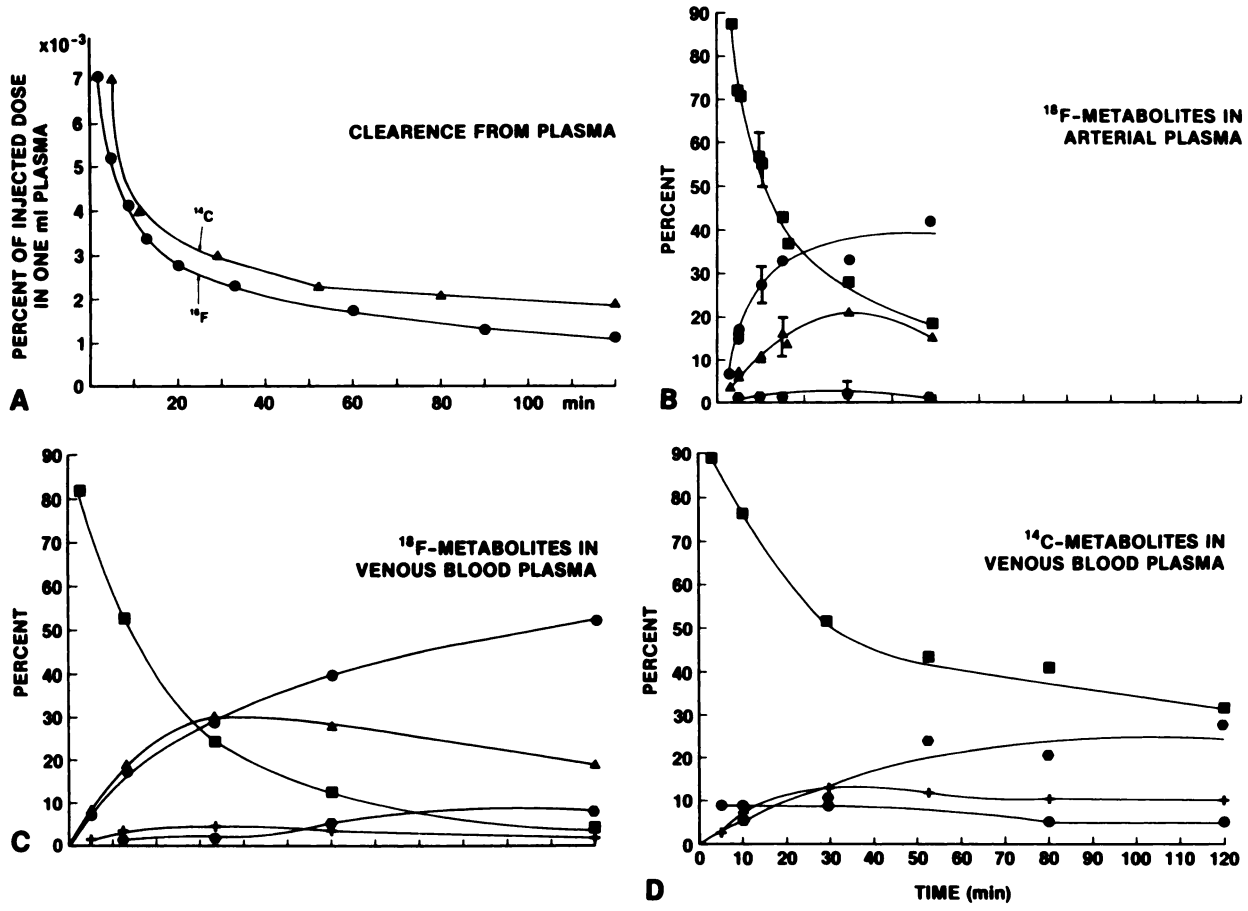

FIGURE 4

Clearance and distribution of ^{14}C and ^{18}F metabolites after an injection of L-[^{14}C]dopa and 6-[^{18}F]fluoro-L-dopa. Panel A, clearance of ^{14}C and ^{18}F from blood plasma in the same individual. Panel B-D, Metabolites after the injection of 6-[^{18}F] fluoro-L-dopa (panel B) and L-[^{14}C]dopa (panel D). (▲) 6-[^{18}F]fluoro-O-sulfato-L-dopa; (●) [^{14}C]dopamine or 6-[^{18}F] fluorodopamine; (●) 3-O-methyl-L-[^{14}C]dopa or 3-O-methyl-6-[^{18}F]fluoro-L-dopa; (+) [^{14}C]homovanillic acid or 6-[^{18}F] fluoro-homovanillic acid. The data in panel B are pooled from blood analysis of nine individuals. The error bars are mean ± 1 s.d.

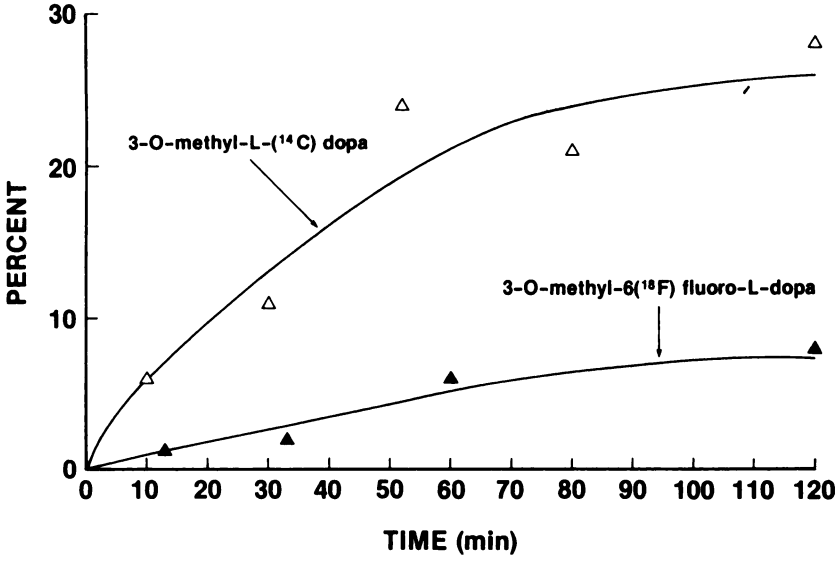

FIGURE 5
Occurrence of O-methyl-metabolites in the human blood after simultaneous injection of L-[^{14}C]dopa and 6-[^{18}F]fluoro-L-dopa measured in the same individual

individual. The concentration of ^{18}F in the striatum after 6-[^{18}F]fluoro-L-dopa was 140 cpm per pixel mCi^{-1}; in the occipital cortex it was 90 cpm per pixel mCi^{-1}.

DISCUSSION

The metabolites of 6-fluoro-L-dopa found in the blood suggest that the metabolic pathways for the 6-fluorinated analog are qualitatively similar to those for L-dopa (*8*). Figure 7 outlines these pathways. There are, however, significant quantitative differences. Whereas O-methylation is the major pathway for L-dopa (Figs. 4D and 5), it only accounts for a few percent for 6-fluoro-L-dopa (Figs. 4B, 4C). It was, therefore, not surprising to find that COMT O-methylates 6-fluoro-L-dopa at 1/4 of the rate of L-dopa (Table 1). We predicted this COMT-inhibiting property of 6-fluoro-dopa (*11, 20*) and more recent kinetic studies in vitro have confirmed that 6-fluoro-L-dopa is a poor substrate for COMT (*21*).

Another metabolic difference between L-dopa and its fluoro-analog that relates to COMT activity is the formation of comparatively large amounts of 3-O sulfato-6-fluoro-L-dopa and fluoro-dopamine. No sulfo-conjugation of L-[^{14}C]dopa was observed. It may be argued that the inhibition of COMT by 6-fluoro-dopa directs the metabolism of 6-fluoro-L-dopa towards the sulfo-conjugation and decarboxylation pathways by mass action (Fig. 7).

TABLE 1
Influence of Fluorine Substitution in L-Dopa on the Rate of O-Methylation by COMT*

Substrate	Rate of O-methylation by COMT nmol/2 hr/ mg protein
L-Dopa	10.7 ± 0.4
5-Fluoro-L-dopa	39.0 ± 1.4
6-Fluoro-L-dopa	2.5 ± 0.2

*Values are mean + s.d., n = 6

The biggest difference between 6-fluorodopa and L-dopa in vivo was the formation of large amounts of 6-[^{18}F]fluorodopamine compared to [^{14}C]dopamine. This suggests that the enzyme aromatic amino acid decarboxylase decarboxylates 6-fluoro-L-dopa faster than dopa. This is in contrast to 5-fluoro-dopa which is decarboxylated with the same kinetics as L-dopa (*22*). A particular perturbation of electron density caused by fluorine in the 6-position of the dopa molecule may be responsible for accelerating the decarboxylation reaction. The molecular mechanism responsible for the difference is under investigation in our laboratory.

Other workers have shown some surprising differences in the biologic properties of isomers of fluorocatechols. For example, 2- and 5-fluorodopamine and dopamine are equipotent in causing renal vasodilation in the dog, whereas 6-fluorodopamine is only 1/4 as active (*23*). Dramatic differences in adrenergic activity also exist among the isomers of fluoronorepinephrine (*24*).

The analytical work described in this paper was done, in part, to determine whether the time-activity curve for ^{18}F in whole venous blood could be used to represent the supply of 6-[^{18}F]fluoro-L-dopa to the brain in a compartmental analysis of the type that has been used successfully to measure regional intracerebral glucose metabolism by ^{14}C- or ^{18}F-labeled deoxyglucose. This method of analysis demands that a kinetic model be constructed. The model can be based either on the movement of the tracer between anatomical compartments, or on the chemical transformation of the tracer

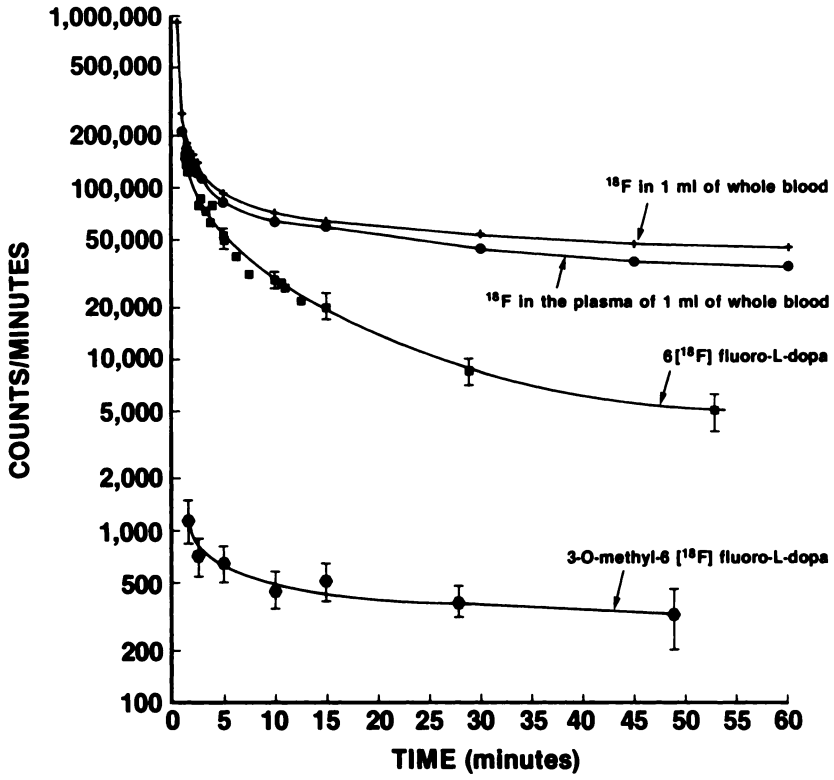

FIGURE 6
Comparison of the ^{18}F disappearance curves on the same scale. Fluorine-18 due to 6-[^{18}F]fluoro-L-dopa in 1 ml of whole blood; ^{18}F due to 3-O-methyl-6-[^{18}F]fluoro-L-dopa in 1 ml of whole blood. The data are pooled from the analysis of nine individuals. The error bars are means ± 1 s.d.

in the organ under investigation; in this case the brain. In either situation the supply to the brain, in our case the time averaged concentration of 6-[^{18}F]fluoro-L-dopa, needs to be known as accurately as possible. As we have shown, venous blood, however easily accessible, will not be sufficient because the time-activity curve for ^{18}F in venous blood differs greatly from that in the arterial blood (Fig. 2). Further, because of metabolism in the periphery, the time course of total ^{18}F in the arterial blood plasma does not represent the "input function" for the mathematical model (Fig. 6). To obtain the "input function" the concentration of 6-[^{18}F] fluoro-L-dopa in the arterial plasma has to be determined in as many sequential blood samples as possible.

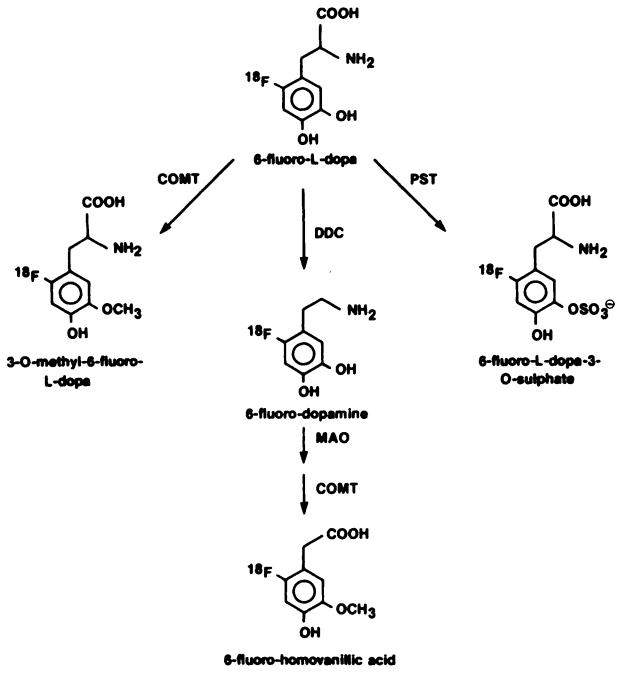

FIGURE 7
Metabolic pathways of 6-[^{18}F]fluoro-L-dopa in the periphery of humans. COMT: catechol-O-methyltransferase; DDC: dopa decarboxylase (aromatic amino acid decarboxylase); PST: phenol sulfotransferase.

Of the five identified metabolites of 6-[^{18}F]fluoro-L-dopa, 3-O-methyl-6-[^{18}F]fluoro-L-dopa is the only one likely to enter the brain because it is known that 3-O-methyl-L-dopa is transported across the blood brain barrier (*25*); the other metabolites are likely to be excluded (*26,27*). Because equivalent i.v. doses of 6-[^{18}F]fluoro-L-dopa and 3 -O-methyl-6-[^{18}F]fluoro-L-dopa produce similar countrates per pixel in the gray structures of the brain at 1 hr and because the time integral of ^{18}F activity in the arterial plasma due to 3-O-methyl-6-[^{18}F]fluoro-L-dopa derived from 6-[^{18}F]fluoro-L-dopa was 1/50 that of the integral derived from 3-O-methyl-6-[^{18}F]fluoro-L-dopa alone, the amount of ^{18}F activity in the brain that might come from metabolically derived O-methylated fluoro-L-dopa can not exceed more than a few percent. Therefore, in studies of cerebral dopamine metabolism involving 6-[^{18}F]fluoro-L-dopa and positron tomography it will be reasonable to assume that the time-activity curve of 6-[^{18}F] fluoro-L-dopa in arterial plasma represents the input function for the brain.

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