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[^{18}F]Fluoro-dopa, an analogue of dopa, and its use in direct external measurements of storage, degradation, and turnover of intracerebral dopamine

(Parkinson disease/schizophrenia)

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ABSTRACT **3,4-Dihydroxy-5-fluorophenylalanine, fluoro-dopa, was injected into rats in which unilateral lesions of the nigrostriatal pathway had been made. The rats rotated towards the side with the lesions, thus providing further evidence that fluoro-dopa is an analogue of dopa. [^{18}F]Fluoro-dopa was then injected intravenously into fully conscious baboons. A well-collimated scintillation detector, aligned along the occipito-mental axis, recorded the accumulation of ^{18}F in the brain. Control animals accumulated ^{18}F continuously for 100 min. This accumulation represents net transport of [^{18}F]fluoro-dopa from blood to brain, decarboxylation to [^{18}F]fluoro-dopamine, storage, and degradation of [^{18}F]fluoro-dopamine. α-Methyl-dopa, a competitive inhibitor of dopa transport and decarboxylation, prevented the accumulation of ^{18}F; reserpine, known to release stored intracerebral dopamine, discharged ^{18}F; pargyline, a monoamine oxidase inhibitor, and haloperidol, a known augmentor of intracerebral dopamine turnover, increased the rate of accumulation of ^{18}F. These changes in the accumulation of intracerebral ^{18}F, after [^{18}F]fluoro-dopa, were commensurate with the known action of the drugs used to induce them and demonstrate the use of a γ-emitting precursor of a neurotransmitter to monitor simply, atraumatically, and externally the intracerebral metabolism of the transmitter in fully conscious primates. When applied to man, the same technique should be able to provide more conclusive evidence than is presently available for the role of catecholamines in schizophrenia and depression. It should also provide further insight into the natural history of nigrostriatal diseases and the action of drugs used in their treatment.**

There is no direct atraumatic method by which the localization or metabolism of intracerebral neurotransmitters can be measured during life in man. However, if a neurotransmitter were labeled with a γ-emitting radionuclide it would be possible, using standard nuclear medicine techniques, to define both the intracerebral distribution and the regional rate of turnover of the molecule. Few of the neurotransmitters cross the blood–brain barrier and, further, the half-lives of the γ-emitting isotopes of their constituent atoms are too short to be used readily.

Because abnormalities of dopamine metabolism are implicated in disorders of locomotion and mood, we synthesized 5-[^{18}F]fluoro-dopa (1). [^{18}F]Fluoro-dopa is a γ-emitting analogue of dopa, the immediate precursor of the neurotransmitter dopamine. Like dopa, [^{18}F]fluoro-dopa is decarboxylated in the brain (2). It produces [^{18}F]fluoro-dopamine. In addition, the rate at which fluoro-dopa is decarboxylated is the same as that at which [^{14}C]dopa is decarboxylated (2). We have also shown that the clearance of [^{18}F]fluoro-dopa from blood is similar to that of [^{14}C]dopa (3) and that the distribution of the metabolites of [^{18}F]fluoro-dopa in the brain of rats is the same as that of the metabolites of [^{14}C]dopa (4). We now present additional *in vivo* evidence that supports the contention that the introduction of a fluorine atom in the 5 position of dopa does not produce a qualitative alteration of the biological behavior of the parent molecule; and, further, we show that [^{18}F]fluoro-dopa can be used to monitor drug-induced alterations of intracerebral dopamine metabolism simply and atraumatically in fully conscious baboons.

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MATERIALS AND METHODS

Response of Rats with Unilateral Lesions in the Substantia Nigra to Fluoro-dopa. Unilateral lesions of the substantia nigra of male Sprague–Dawley rats, 250–300 g, were made with 6-hydroxy-dopamine according to the method of Ungerstedt (5). After recovery each rat was treated with carbidopa (100 mg/kg of body weight) and placed in a round glass jar, 30-cm diameter. The rate at which the rats circled in the jar was recorded visually during a control period, after DL–dopa (100 mg/kg), and after apomorphine (10 mg/kg). Only rats that circled in response to both DL-dopa and apomorphine were used in subsequent experiments. These rats were then given intraperitoneally fluoro-dopa, 230 mg/kg, dissolved in saline with 1% ascorbic acid, and the rate at which each rat rotated was recorded for 1 hr after it had begun to circle. The dose of fluoro-dopa was chosen to be equivalent to 100 mg of dopa per kg on the assumption that at physiological pH approximately half of the fluoro-dopamine derived from fluoro-dopa would be un-ionized whereas almost all of the dopamine made from dopa would be in this form; pK_a of the 4-hydroxyl group of fluoro-dopamine is 7.42 (6), pKa of the 4-hydroxyl group of dopamine is 8.90 (7). In a further experiment the rats were pretreated with haloperidol, 3 mg/kg, and then given a second intraperitoneal injection of fluoro-dopa. Again the rate at which each rotated was recorded.

Drug-Induced Changes of Intracerebral Dopamine Metabolism. Mature female baboons (*Papio papio*) weighing 7–13 kg were obtained from Primate Imports Corp., Port Washington, Long Island, NY. They were trained over a period of 3 weeks to sit quietly, without tranquilizers, in a restraining chair.

A well-collimated 5 cm × 5 cm NaI(Tl) scintillation detector was aligned along the occipito-mental axis so that the sensitive volume of the detector measured only radioactivity contained in the head. The output from the detector was analyzed by γ spectroscopy and traced on a strip chart recorder.

3,4-Dihydroxy-5-[^{18}F]fluorophenylalanine, [^{18}F]fluoro-dopa, specific activity 2–100 μCi/mg, was synthesized by the method of Firnau *et al.* (1) and purified by reverse-phase high-pressure liquid chromatography. [^{18}F]Fluoro-dopa for intravenous in-

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Table 1. Drug schedules used in baboon experiments

Type of study	Dose of drug, mg/kg	DL-[^{18}F]fluoro-dopa Intravenous administration: Bolus, μCi	Infusion: Rate, μCi/min	Infusion: Duration, min	Specific activity, μCi/mg
Control	None	80	—	—	16
α-Methyl-dopa, 1 hr*	200	65	—	—	13
α-Methyl-dopa, 3 hr*	200	830	—	—	166
Control	None	85	—	—	17
Reserpine	3.5	557	—	—	111
Reserpine	4.5	764	—	—	153
Control	None	40	0.10	100	10
Pargyline	90	80	0.25	80	20
Pargyline	90	78	0.58	80	25
Control	None	50	0.45	110	22
Haloperidol	1.3	25	0.36	70	10
Haloperidol	1.3	150	2.30	70	62
Haloperidol	1.0	95	1.36	70	37

Doses of drugs and DL-[^{18}F]fluoro-dopa used in external measurements of storage, degradation, and turnover of intracerebral dopamine. All amounts of radioactivity are corrected to the time at which the bolus of [^{18}F]fluoro-dopa was injected. α-Methyl-dopa injection was Aldomet, 50 mg/ml (Merck, Sharp and Dohme). Reserpine injection was Serpasil, 2.5 mg/ml (Ciba). Pargyline injection was in saline, 50 mg/ml (Abbott). Haloperidol injection was Haldol, 2.0 mg/ml (McNeil).

* Interval between administration of α-methyl-dopa and [^{18}F]fluoro-dopa.

jection was dissolved in 0.05 M sodium phosphate buffer, pH 3.5, containing ascorbic acid at 3 mg/ml.

Blood Background Subtraction. In order to monitor changes in intracerebral ^{18}F content resulting only from [^{18}F]fluoro-dopa metabolism, it was necessary to subtract the contribution that ^{18}F in the blood in the head made to the total radioactivity recorded. The intracranial blood volume was estimated by injecting ^{113m}In-labeled transferrin intravenously and measuring the amount of indium seen by the head probe and the concentration of indium in a peripheral blood sample. ^{113m}In-transferrin was prepared by incubating baboon serum for 5 min with $^{113m}InCl_3$ (^{113}Sn–^{113m}In generator obtained from Union Carbide Corp.). The amount of ^{113m}In in the blood was measured with a lithium drifted germanium solid-state detector. The ^{113m}In activity in the blood and ^{113m}In activity in the head probe were used to estimate the intracranial blood volume. The ^{18}F content of the intracranial blood pool could then be calculated at any time after the injection of [^{18}F]fluoro-dopa by multiplying the concentration of ^{18}F in the peripheral blood by the estimated intracranial blood volume. ^{18}F in the peripheral blood was measured with the solid-state detector. The intracerebral ^{18}F activity due to fluoro-dopa and its metabolites was then obtained by subtracting the ^{18}F content of the intracranial blood pool from the total ^{18}F recorded from the head. All ^{18}F and ^{113m}In data were corrected for radioactive decay.

The effects on the intracerebral accumulation of ^{18}F of α-methyl-dopa, reserpine, pargyline, and haloperidol were studied as outlined below. Table 1 summarizes the drug schedules and mode of administration of [^{18}F]fluoro-dopa.

α-Methyl-dopa. A control experiment was done in which the rate of accumulation of ^{18}F in the brain was recorded for 90 min after a bolus injection of [^{18}F]fluoro-dopa. In a second experiment a baboon was given α-methyl-dopa 1 hr before [^{18}F]fluoro-dopa and in a third experiment α-methyl-dopa was given 3 hr before [^{18}F]fluoro-dopa.

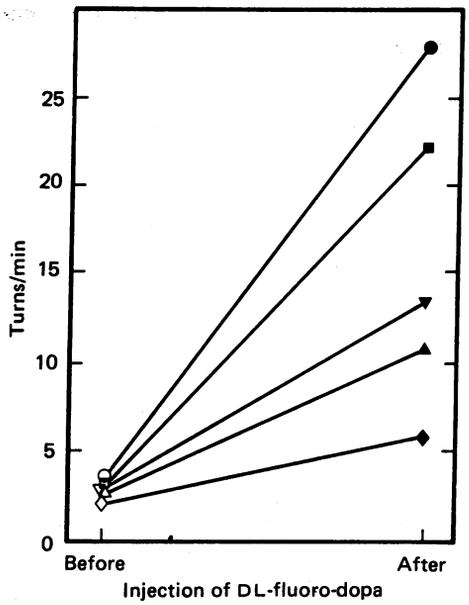

FIG. 1. Circling behavior induced by intraperitoneal DL-fluoro-dopa, 230 mg/kg, in rats with unilateral lesions of substantia nigra. Each animal acted as its own control; open symbols represent control experiments, closed symbols represent effects of DL-fluoro-dopa.

Reserpine. A control experiment was done as described above. In two other experiments reserpine was injected intravenously 30 min after the bolus injection of [^{18}F]fluoro-dopa.

Pargyline. A control experiment was done in which a bolus injection of [^{18}F]fluoro-dopa was given at zero time, followed by a continuous infusion of [^{18}F]fluoro-dopa that was started at 10 min. A single intravenous injection of pargyline was given at 40 min.

Haloperidol. The protocol used in haloperidol experiments was the same as that described for pargyline. A single intravenous injection of haloperidol was given at 45 min.

RESULTS

Response of Rats with Unilateral Lesions in the Substantia Nigra to Fluoro-dopa. When fluoro-dopa, 230 mg/kg, was given all of the treated rats circled away from the side of the lesion (Fig. 1). The dose of fluoro-dopa used in these experiments was that which produced a rate of turning similar to that seen after DL-dopa, 100 mg/kg. When haloperidol, 3.0 mg/kg, was given 30 min before fluoro-dopa, the rats did not circle and their behavior was indistinguishable from that seen in control experiments in which no drugs were administered.

Drug-Induced Changes of Intracerebral Dopamine Metabolism in Baboons. When *α-methyl-dopa*, 200 mg/kg, was given 3 hr before [^{18}F]fluoro-dopa, ^{18}F accumulated continuously in the brain for 2 hr but the amount of this accumulation was less than that seen in the control experiment. When α-methyl-dopa was given 1 hr before [^{18}]fluoro-dopa, ^{18}F accumulated for approximately 5 min. Thereafter, there was a progressive decrease in the amount of ^{18}F retained in the brain (Fig. 2).

When *reserpine*, 3.5 mg/kg, was given 30 min after [^{18}]-fluoro-dopa, ^{18}F continued to accumulate in the brain for a further 15 min and then fell (Fig. 3). A similar result was seen in another baboon.

When *pargyline*, 90 mg/kg, was given 40 min after a bolus injection of [^{18}F]fluoro-dopa (i.e., 30 min after start of the

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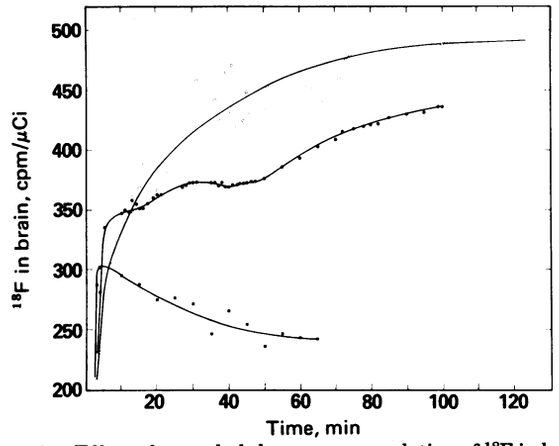

FIG. 2. Effect of α-methyl-dopa on accumulation of ^{18}F in brain. In each experiment [^{18}F]fluoro-dopa was injected at zero time. ○, Control, no α-methyl-dopa given; ■, α-methyl-dopa, 200 mg/kg, intravenously 1 hr before [^{18}F]fluoro-dopa; ●, α-methyl-dopa, 200 mg/kg, intravenously 3 hr before [^{18}F]fluoro-dopa. In this and the following figure, ^{18}F accumulation is given in terms of cpm/μCi of [^{18}F]fluoro-dopa injected.

infusion of [^{18}F]fluoro-dopa), the rate at which ^{18}F accumulated in the head was greater than that of the control (Fig. 4). A similar result was seen in a second baboon.

When *haloperidol*, 1.3 mg/kg, was given 45 min after a bolus injection of [^{18}F]fluoro-dopa (i.e., 35 min after start of infusion of [^{18}F]fluoro-dopa), the rate at which ^{18}F accumulated in the head was greater than that of the control (Fig. 5). A similar result was seen in two other baboons.

DISCUSSION

When unilateral lesions of the substantia nigra are made in rats by the local injection of 6-hydroxy-dopamine, the animals turn away from the side of the lesion when they are given L-dopa, the precursor of dopamine. It is believed that this response is due to supersensitivity of synapses in the ipsilateral corpus striatum and is specific for agonists of dopamine (5). In the present study 5-fluoro-dopa produced contralateral circling in rats with such lesions. Because fluoro-dopa is known to be decarboxylated to fluoro-dopamine (3), this observation supports our finding that fluoro-dopa causes arousal in mice treated with reserpine (4). Together the results provide compelling evidence that fluoro-dopamine is an agonist of dopamine. The implications of this are 2-fold. First, the presence of a fluorine atom in the 5 position of dopamine appears not to influence its agonistic properties. Second, ^{18}F-labeled dopa, the precursor of [^{18}F]-fluoro-dopamine, can be used to monitor intracerebral dop-

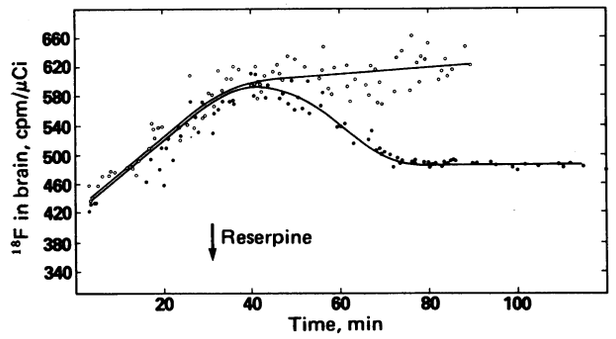

FIG. 3. Effect of reserpine on accumulation of ^{18}F in brain. In each experiment [^{18}F]fluoro-dopa was injected at zero time. ○, Control, no reserpine given; ●, reserpine, 3.5 mg/kg, was injected intravenously at 30 min.

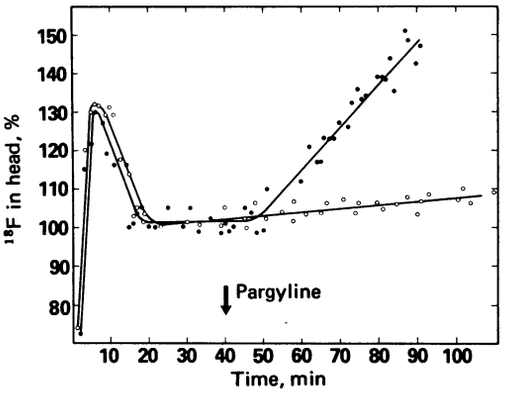

FIG. 4. Effect of pargyline on accumulation of ^{18}F in head. In each experiment a bolus of [^{18}F]fluoro-dopa was injected at zero time and [^{18}F]fluoro-dopa was continuously infused from 10 min. ○, Control, no pargyline given; ●, pargyline, 90 mg/kg, intravenously at 40 min. ^{18}F is expressed as percent of 18-min value.

amine metabolism simply and atraumatically with scintillation probes placed externally over the head. This latter potential was explored by manipulating pharmacologically the intracerebral dopamine metabolism of baboons and observing externally the pattern of accumulation of ^{18}F in their brains.

When [^{18}F]fluoro-dopa is injected as a bolus, we postulate that ^{18}F accumulates continuously in the brain (Figs. 2 and 3) as the net result of transport of [^{18}F]fluoro-dopa across the blood–brain barrier, decarboxylation of [^{18}F]fluoro-dopa to [^{18}F]fluoro-dopamine, storage of [^{18}F]fluoro-dopamine in synaptic vesicles, and degradation of [^{18}F]fluoro-dopamine. Dopa decarboxylase is known to be present in the endothelial cells of intracerebral capillaries in rodents, but Langelier *et al.* (8) could not find any decarboxylase activity in the cerebral capillaries of primates. Thus, the ^{18}F that we observed in the brains of baboons does not result from labeled [^{18}F]fluoro-dopamine trapped in the enzymatic blood–brain barrier.

α-Methyl-dopa, a known inhibitor of dopadecarboxylase at the concentrations used in our experiment (9), will also inhibit the transport of [^{18}F]fluoro-dopa across the blood–brain barrier because the K_m for the carrier-mediated transport of neutral amino acids is 0.1 mM (10). When [^{18}F]fluoro-dopa was injected into a baboon 1 hr after α-methyl-dopa, the small transient accumulation of ^{18}F in the brain probably resulted from a fraction of the dose of [^{18}F]fluoro-dopa that entered the brain but could not be decarboxylated. The subsequent reduction in the amount of ^{18}F probably resulted from the return of unde-

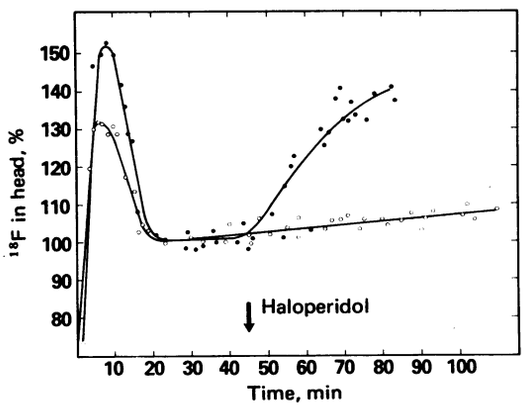

FIG. 5. Effect of haloperidol on accumulation of ^{18}F in head. In each experiment a bolus of [^{18}F]fluoro-dopa was injected at zero time and [^{18}F]fluoro-dopa was continuously infused from 10 min. ○, Control, no haloperidol given; ●, haloperidol, 1.3 mg/kg, intravenously at 45 min. ^{18}F is expressed as percent of 18-min value.

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carboxylated [^{18}F]fluoro-dopa to the blood. When [^{18}F]fluoro-dopa was injected 3 hr instead of 1 hr after α-methyl-dopa, a larger amount of ^{18}F accumulated in the brain. This was to be expected because it is known that in rats the effect of α-methyl-dopa lasts for only 3 hr (9).

Reserpine destroys the ability of neurones to store dopamine (11); it does not interfere with the metabolism of this amine (12). The fall in ^{18}F activity in the brain that began soon after the administration of reserpine (Fig. 3) presumably resulted from a release of [^{18}F]fluoro-dopamine that had been formed and stored within 30 min of the administration of precursor [^{18}F]-fluoro-dopa. Because [^{18}F]fluoro-dopa will, like dopa, be *O*-methylated in noncerebral tissues, particularly the liver, and returned to the blood, some of the ^{18}F activity that was in the brain when reserpine was given will have been due to 3-*O*-methyl[^{18}F]fluoro-dopa. The biological half-life of 3-*O*-methyl-dopa in the brain is approximately 13 hr (13), and thus the amount of 3-*O*-methyl[^{18}F]fluoro-dopa that was in the brain when reserpine was given would remain relatively unchanged during the course of our observations. This would explain the retention of the ^{18}F in the brain depicted in the latter part of Fig. 3. Further, because DL-[^{18}F]fluoro-dopa was injected, some of the activity retained in the brain will also have been due to D-[^{18}F]fluoro-dopa as well as D-3-*O*-methyl[^{18}F]fluoro-dopa (14).

Pargyline, an inhibitor of monoamine oxidase, with a rapid onset of action (15), was used to prevent the degradation of [^{18}F]fluoro-dopamine. In a typical experiment, half of the [^{18}F]fluoro-dopa was injected at zero time and the remainder was infused over the interval 10–100 min. This was done in an attempt to maintain the concentration of [^{18}F]fluoro-dopa in blood. It was known that less than 1% of a spike injection of [^{18}F]fluoro-dopa remained in the circulation at 20 min. Consequently, it would have been very difficult to demonstrate a pargyline-induced increase in the accumulation of ^{18}F in the head if at the time pargyline was given there was so little tracer in the circulation. The result of this particular mode of administration of [^{18}F]fluoro-dopa is shown in the control curve of Fig. 4. The initial hump is due to a combination of the convolution of the initial spike injection with the impulse response of the brain and the infusion which began at 10 min. The presentation in Fig. 4 also differs from that used in the α-methyl-dopa and reserpine experiments in that blood background has not been subtracted and each data point has been normalized to the 18-min value.

We interpret the rapid rise in the rate of accumulation of ^{18}F that occurred when pargyline was injected to be due to freshly formed [^{18}F]fluoro-dopamine that cannot be degraded.

Haloperidol blocks postsynaptic dopaminergic receptors and as a consequence increases the rate of turnover of intracerebral dopamine and the production of phenolic acids (16). The immediate and marked increase in the intracerebral accumulation of ^{18}F that occurred when haloperidol was injected (Fig. 5) was presumed to result primarily from the accumulation of ^{18}F-labeled phenolic acids. This result was particularly gratifying because it showed that [^{18}F]fluoro-dopa and an externally placed probe could be used to detect changes in the rate of turnover of neurotransmitter that had been brought about by direct modification of the postsynaptic receptor site.

The results of these pharmacological challenges confirm that changes in the transport across the blood–brain barrier and decarboxylation of dopa, the storage, the oxidative deamination, and the turnover of intracerebral dopamine can each be monitored by a scintillation probe placed over the head when [^{18}F]fluoro-dopa is used as a marker for native dopa.

To date we have made qualitative assessments of changes of intracerebral dopamine metabolism. However, we believe that [^{18}F]fluoro-dopa will enable quantitative measurements of regional dopamine metabolism to be made in man by combining the mathematical approach of Raichle *et al.* (17) with rapid sequential measurements of regional intracerebral radioactivity made with positron emission tomographic devices. Further, we suggest that intracerebral noradrenalin and serotonin metabolism will also be accessible to similar investigative approaches.

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