Intracerebral dopamine metabolism studied by a novel radioisotope technique

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Disorders of locomotion and mood are associated with disturbances of intracerebral catecholamine metabolism. Ehringer & Hornykiewicz (1960) showed that there is an absolute deficiency of dopamine in the caudate nucleus of patients with Parkinson's disease, but no such clear cut relation has been established for other disorders of locomotion or for disorders of mood.

The purpose of this paper is to present the results of a series of experiments which show that [^{18}F]fluoro-dopa is a radioactive analogue of dopa which can be injected intravenously and used to study intracerebral dopamine metabolism in man by means of routine non invasive isotopic procedures.

[^{18}F] 3,4-Dihydroxy-5-fluorophenylalanine, fluoro-dopa, was synthesized by the method of Firnau, Nahmias & Garnett (1973). The specific activity of this material ranged from 2 to 100 μCi mg^{-1}. DL-3,4-dihydroxyphenylalanine [alanine-2-^{14}C] (256 μCi mg^{-1}) [^{14}C] dopa, and DL-3,4-dihydroxy-5-fluorophenylalanine [alanine-2-^{14}C] hydrobromide (39 μCi mg^{-1}) were obtained from New England Nuclear, Boston, Mass.

When [^{18}F]fluoro-dopa (25 mg kg^{-1}) or [^{14}C]dopa (25 mg kg^{-1}) was injected into mature rats and the rats were killed at 1 or 2 h, the intracerebral distribution of the labelled analogues was as shown in Table 1. The metabolites derived from fluoro-dopa were separated into two fractions: amino acids and catecholamines (Bartholini & Pletscher, 1968). The relative distribution of these fractions within the brain was measured 30 min after injection (Table 2).

Carlsson, Lindqvist & Magnusson (1957) concluded that the arousal produced by giving dopa to reserpinized animals was due to an amine formed from dopa.

Table 1. *Intracerebral distribution of ^{18}F and ^{14}C in rats after simultaneous injection of* DL-[^{18}*F]fluoro-dopa and* DL-[^{14}*C] dopa.* Each result is the average of 3 studies (with one standard deviation) and represents radioactivity per g brain part expressed as percentage of radioactivity per g cerebral cortex. Brains dissected according to Glowinski & Iversen (1966).

Brain areas	Time after injection of ^{18}F-fluoro-dopa		Time after injection of ^{14}C-dopa	
	1 h	2 h	1 h	2 h
Cerebral cortex	100	100	100	100
Caudate nucleus	113 s.d. 9	161 s.d. 60	129 s.d. 11	138 s.d. 20
Hypothalamus	95 s.d. 8	143 s.d. 78	116 s.d. 8	114 s.d. 3
Cerebellum	88 s.d. 22	105 s.d. 40	100 s.d. 8	96 s.d. 10
Midbrain	83 s.d. 9	88 s.d. 25	94 s.d. 12	96 s.d. 18

* Correspondence.

When we gave fluoro-dopa to reserpinized mice a similar arousal was observed. Twelve male mice were studied. Reserpine (15 mg kg^{-1}) was injected intraperitoneally and 19 h later the mice were torpid and dishevelled and had developed complete ptosis. Four mice were then given fluoro-dopa (600 mg kg^{-1}) intraperitoneally, four were given dopa in the same dose and the remaining four served as controls and were given saline only. After 2 min, the mice which had been given fluoro-dopa or dopa became hyperactive. They ran about or groomed incoordinately and, on a number of occasions leapt several centimetres into the air. During this period of arousal the ptosis disappeared. No distinction could be made between the mice given fluoro-dopa and those given dopa. The arousal lasted for approximately 20 min, after which the animals gradually sank again into a torpid state. No arousal occurred in the mice which had received saline.

The effect of reserpine on the intracerebral accumulation of [^{18}F]fluoro-dopa was next studied in a conscious female baboon. The baboon was trained over a period of three weeks to sit quietly, without tranquillizers, in a restraining chair. She was then studied on four separate occasions. On two of these she was pre-treated with reserpine until ptosis occurred (3 mg kg^{-1} over 24 h). On each occasion 400 μCi [^{18}F]fluoro-dopa (6–80 μCi mg^{-1}) was injected intravenously and the radioactivity in the head was recorded for 60 min by means of a collimated 2″ NaI(Tl) detector connected through a pulse height analyser to a strip chart recorder. Blood samples were taken during each study to establish the pattern of disappearance of ^{18}F from the blood. Indium-113m transferin was used to establish the size of the intracranial blood pool relative to the size of a blood sample. From the size of this pool, the blood clearance data and a geometrical factor relating ^{18}F to ^{113m}In, the contribution of ^{18}F in the intracranial blood pool at any time could be calculated. This contribution was subtracted from the total ^{18}F activity recorded from the head. All of the ^{18}F data were corrected for radioactive decay. Fig. 1 shows the time course of accumulation of ^{18}F in the head. It should be stressed that the difference between the non-reserpinized and the reserpinized animal was apparent before correction for ^{18}F contained in the intracranial blood pool.

Table 1 shows that the intracerebral distribution of fluoro-dopa is the same as that of dopa while Table 2 shows that the intracerebral distribution of fluorinated

Table 2. *Intracerebral distribution of metabolites of [^{14}C] dopa and [^{14}C] fluoro-dopa.* The concentration (% dose g^{-1}) of amines (A) is expressed as percentage of the concentration of the labelled amino acids (AA) in the same brain part. The low specific activity of DL-fluoro-dopa necessitated the use of the larger quantity of this material.

Brain areas	DL-dopa* Rabbit 30 min 1·5 mg kg^{-1} A/AA†	DL-F-dopa Rat 30 min 3·15 mg kg^{-1} A/AA†
Cortex	19	16 s.d. 3
Caudate nucleus	180	130 s.d. 27
Medulla oblongata	51	40 s.d. 10
Cerebellum	8	12 s.d. 5

* Taken from Pletscher & Gey (1962).
† Ratio of catecholamines to amino acids.

amines is similar to that of the amines derived from dopa (Pletscher & Gey, 1962). The high relative concentration of fluorinated amines in the caudate nucleus supports the proposition that the addition of fluorine in the 5-position on the aromatic ring of dopa does not substantially alter the intracerebral behaviour of dopa. This proposition is strengthened by *in vitro* experiments in which it has been shown that fluorodopamine is produced from fluoro-dopa by brain dopa decarboxylase at the same rate as dopamine is produced from dopa (Firnau, Garnett & others, 1976). It is further strengthened by the observation that fluoro-dopa produces a transitory reversal of the effect of reserpine in mice.

Some of the biochemical and biological properties of fluoro-dopa having been established, the experiment on the baboon was designed to show that the effect of the administration of reserpine, a manouvere known to reduce dopamine accumulation in the brain, could be detected from changes in the amount of ^{18}F present in the head after an intravenous injection of [^{18}F] fluoro-dopa. Fig. 1 shows that pre-treatment with reserpine produces a strikingly different pattern of intracerebral accumulation of ^{18}F from that of the control. The progressive increase in the amount of ^{18}F recorded from the head in the control studies suggests the formation and storage of fluorodopamine.

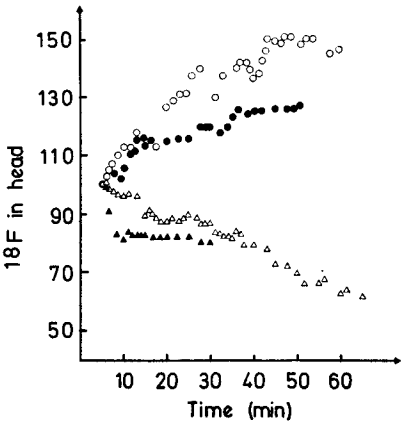

FIG. 1. The ordinate in each experiment represents the intracerebral accumulation of ^{18}F expressed as a percentage of the 5 min value. [^{18}F]Fluoro-dopa injected at zero time. Triangles represent studies in reserpinized (3 mg kg^{-1}) baboon; circles represent studies in control baboon.

The initial rise in ^{18}F which occurred in the reserpinized animal probably represents a rapid transfer of [^{18}F] fluoro-dopa from the blood to the brain. Since reserpine only inhibits the storage of dopamine (Bertler, 1961) no further accumulation of ^{18}F as fluorodopamine was possible. [^{18}F]Fluoro-dopa, or its metabolites, were therefore cleared from the brain and the radioactivity recorded from the head fell.

The implications of these results are that [^{18}F]fluoro-dopa can be used to study intracerebral dopamine metabolism in man as well as in other primates provided that it is proved that the addition of fluorine to the dopa-molecule does not produce any untoward toxic effects. It is suggested that a protocol, similar to that described here in the baboon experiments, could be used to investigate the role of dopamine in disorders of locomotion and affect. It could also be used to examine the dopamine aspect of the pharmacology of the wide variety of psychotropic drugs presently marketed.

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