[^{18}F] Fluoro-Dopa: a Unique Gamma Emitting Substrate for Dopa Decarboxylase

The brain scanning agents formulated so far have not been specific for either normal or diseased brain tissues. Nor has a radiopharmaceutical been synthesized which would allow its localization in the various brain regions by a simple procedure such as scanning. Similarly, there is no simple method by which intracerebral catecholamine metabolism may be investigated. However, it seemed that if a suitable gamma emitting label could be attached to dopamine it might be possible to investigate the metabolism of the basal ganglia by routine procedures of nuclear medicine. Since dopamine itself does not enter the brain its immediate precursor, Dopa, which does cross the blood brain barrier, was labelled isotopically. The gamma emitting isotopes of the constituents of Dopa have very short half lives and cannot readily be used. Instead, [^{18}F] fluoro-Dopa was synthesized[1]. ^{18}F has a half life of 1.8 h which allows tests of up to 4 h without unreasonable risks of irradiation.

The present study was designed to find out how new compound was handled by the enzyme dopa decarboxylase (EC 4.1.1.26). The product of decarboxylation was identified, the kinetics of the reaction were measured and the effects of adding the coenzyme, pyridoxal-5′-phosphate (PLP), or the inhibitor, α-methyl-Dopa, were investigated.

Materials and methods. [^{14}C] L-Dopa and DL-5-fluoro-Dopa [2-alanine-^{14}C]. HBr (New England Nuclear Corp., Boston, Mass.) with specific activities of 21 mCi/mmol and 11.74 mCi/mmol respectively were used. The Dopa was diluted for our experiments to 0.195 mCi/mmol. [^{18}F] DL-5-Fluoro-Dopa was prepared and purified as previously described[1]. Dopa decarboxylase was contained in homogenates of hog kidney, hog brain and rabbit brain. The fresh tissues were homogenized in ice-cold 0.2 *M* Na-phosphate buffer at pH 6.8 (1 part tissue: 3 parts buffer) and then centrifuged at 100,000 *g* for 1 h. The protein content of the enzyme-containing high speed supernatants (hss) from the hog kidney and the hog brain were 26.7 mg/ml and 4.4 mg/ml, respectively.

The enzyme preparation (hss) together with pyridoxal-5′-phosphate (PLP) was preincubated for 10 min with either iproniazid phosphate or nialamide before the substrate was added. The reaction mixtures were incubated at 37 °C for up to 2 h. The reaction was stopped by heating to 100 °C for 1 min and the heat-precipitated proteins were centrifuged down.

When ^{14}C substrates, [^{14}C] L-Dopa or [^{14}C] DL-fluoro-Dopa were used the amines produced by decarboxylation were separated on Amberlite CG-50[2] and their ^{14}C content was measured in a toluene-Triton X solution containing scintillator. The ^{14}C amines were also chromatographed on paper (Whatman No. 20; MeOH:*n*-BuOH:AcOH:H_2O, 4:3:2:1, 16 h) and the position of the radioactivity determined. Any O-methylated phenylethylamines which may have been produced by catechol O-methyltransferase in the homogenates were separated on alumina[3].

When [^{18}F] fluoro-Dopa was the substrate the amine was separated on Dowex 50–X8[3] and its ^{18}F content measured. The amine was also examined by thin-layer chromatography (Silica Gel; *n*-BuOH:AcOH:H_2O, 4:1:1; 1% *p*-nitro-benzene diazonium tetrafluoroborate).

[^{14}C] Fluoro-Dopa and [^{18}F] fluoro-Dopa were each used to determine the Michaelis-Menten constant. The conditions under which these determinations were made are shown in Table I.

Results. The chromatographic identification of the product of decarboxylation of fluoro-Dopa. The ratio Rf [^{14}C] F-Dopa to Rf Dopa was 1.16 ± 0.04 (paper chromatography). The ratio Rf [^{18}F] F-Dopa to Rf Dopa was 1.13 ± 0.04 (thin layer chromatography). The same ratio was obtained from the Rf values of fluorinated and authentic dopamine: thus, ratio Rf [^{14}C] F-dopamine to Rf dopamine was 1.11 ± 0.04 (paper chromatography) and the ratio Rf [^{18}F] F-dopamine to Rf dopamine was 1.12 ± 0.04 (thin layer chromatography). A single peak of ^{14}C or ^{18}F always occurred at the position of the putative fluorinated amine. Further, no *O*-methylated amines were ever found.

Kinetics. When fluoro-Dopa was the substrate and enzyme from hog kidney was used the rate of reaction was 10.7 nmol (mg protein)$^{-1}$ min^{-1}, but when enzyme from hog brain was used the rate of reaction was 0.202 nmol (mg protein)$^{-1}$ min^{-1}. The K_m value for fluoro-Dopa (both radioisomers, Table I) with the hog kidney enzyme was 1.66 ± 0.364 nmol/l (mean ± standard deviation, 6 determinations). It was 3.0 mmol/l for L-Dopa (Table I). The K_m for fluoro-Dopa with hog brain dopa decarboxylase was not significantly different from that for kidney ($p > 0.05$).

[1] G. FIRNAU, C. NAHMIAS and S. GARNETT, J. appl. Radiat. Isotopes *24*, 182 (1973).
[2] F. JAVOY, H. HARMON and J. GLOWINSKI, Europ. J. Pharmac. *10*, 178 (1970).
[3] G. BARTHOLINI and A. PLETSCHER, J. Pharmac. exp. Ther. *161*, 14 (1968).

Table I. Experimental conditions for K_m determinations

	Hog kidney cortex			Hog brain	
Substrates	[^{14}C] F-Dopa	[^{18}F] F-Dopa	Dopa	[^{14}C] F-Dopa	Dopa
Concentration range (μmol/l)	85–319	26–260	256–1020	85–341	256–1020
hss (ml)	0.1	0.5	0.1	0.5	0.2
Protein in hss (mg)	2.68	13.0	2.68	2.19	0.88
PLP (μmol/l)	30	106	30	30	30
Iproniazid phosphate (μmol/l)	100	—	100	100	100
Nialamide (μmol/l)	—	320	—	—	—
Total volume (ml)	1.0	1.52	1.0	1.0	1.0
Time of incubation (min)	10	18	10	20	20
K_m (mmol/l)	1.68 ± 0.436[a]	1.82; 1.40	3.0	2.31	3.0

Final concentrations are given. The concentrations for fluoro-Dopa are half the actual concentration of the racemic mixture. [a] Mean ± standard deviation (4 determinations).

Stimulation and inhibition. The rate of decarboxylation of fluoro-Dopa was increased by PLP (Table II). It was decreased by α-methyl-Dopa (Table III).

Discussion. Our observations show that fluoro-Dopa is decarboxylated to fluoro-dopamine by dopa decarboxylase, an enzyme known to have a broad specificity[4]. As might be expected the rate of this reaction was increased by the addition of PLP, the coenzyme for dopa decarboxylase[5] and inhibited by α-methyl-Dopa, a known competitive inhibitor[6].

The K_m value for fluoro-Dopa was slightly lower than, but of the same order as that obtained with Dopa. Our estimate of the K_m for the latter compound was similar to that reported by HARTMAN et al.[7] and YUWILER et al.[8]. It is concluded that [^{18}F] fluoro-Dopa is an analogue of Dopa which could be used to investigate the intracerebral metabolism of Dopa and dopamine. Such investigations could make use of peripheral decarboxylase inhibitors to enhance the penetration of the gamma-emitting analogue into the brain.

Table II. Stimulation of fluoro-Dopa decarboxylation by pyridoxal-5′-phosphate (PLP)

PLP (μmol)	Fluoro-dopamine formed (nmol)
0	6.06
10	25.9
30	24.4

Incubation conditions: 0.1 ml hss of hog kidney cortex (equivalent to 2.69 mg protein); iproniazid phosphate, 10^{-5} mol/l; fluoro-Dopa (as the L-isomer), 1.28×10^{-4} mol/l; in a volume of 1.0 ml/10 min at 37°.

Table III. Inhibition of decarboxylation by α-methyl-Dopa

Enzyme preparation from rabbit brain	α-Me-Dopa	Fluorodopamine (μmol) formed (g wet wt. of brain)$^{-1}$ h^{-1}
crude	—	3.72
crude	+	0.12
hss	—	2.30
hss	+	0.32

Incubation conditions: Enzyme preparation (corresponding to 0.5 g of brain tissue, wet weight), 2.0 ml; PLP, 5×10^{-5} mol/l; nialamide, 2.14×10^{-5} mol/l; fluoro-Dopa (as L-isomer), 1.41×10^{-4} mol/l; L-α-methyl-Dopa, 2.38×10^{-4} mol/l (final concentrations), in a volume of 3.12 ml.; 1 h at 37 °C. The amounts of fluorodopamine tabulated have been corrected for non-enzymic decarboxylation.

Summary. [^{18}F]-5-fluoro-Dopa is a substrate for dopa decarboxylase of kidney and brain. Its potential use in brain studies is proposed.

G. FIRNAU, E. S. GARNETT, T. L. SOURKES[9] and K. MISSALA[9]

McMaster University Medical Centre, Hamilton 16 (Ontario, Canada), and Faculty of Medicine, McGill University, Montreal (Québec, Canada), 13 June 1975.

[4] W. LOVENBERG, H. WEISSBACH and S. UDENFRIEND, J. biol. Chem. *237*, 89 (1962).
[5] D. E. GREEN, L. F. LELOIR and V. NOCITO, J. biol. Chem. *161*, 559 (1945).
[6] T. L. SOURKES, Arch. Biochem. Biophys. *51*, 444 (1954).
[7] W. J. HARTMAN, R. I. AKAWIE and W. C. CLARK, J. biol. Chem. *216*, 507 (1955).
[8] A. YUWILER, E. GELLER and S. EIDUSON, Arch. Biochem. Biophys. *89*, 143 (1960).
[9] Faculty of Medicine, McGill University, Montréal, Québec, Canada.

Synthesis of Histidine^2-Angiotensin II Analogues[1]

Histidine^2-angiotensin II, an analogue of Ile^5-angiotensin II, was found essentially inactive as a pressor agent, but it displayed minor oxytocic activity[2]. For the purpose of comparison, it seemed worthwhile to determine the effects of a protected imidazole nucleus and the β-aspartyl bond of the aforementioned analogue, since Asp^1-β-Val^5-angiotensin II shows an increased and prolonged activity[3]. Towards this end, synthetic steps were conducted in part by stepwise addition via the carbodiimide method or by fragment condensation via the azide route in order to avoid the danger of racemization[4]. Thus, Z-Val-Tyr-Ile-$NHNH_2$ (I), prepared from the corresponding methyl ester[5], was coupled via the azide method with H-(im-Bzl)His-Pro-Phe-OCH_3 dihydrobromide[6], desalted, prior its use, by column chromatography on DOWEX 2-X8 using MeOH as an eluent. The resulting hexapeptide, Z-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OCH_3 (II) gave comparable or higher values than those reported[7]. Compound II was subjected to selective catalytic hydrogenation[8] over palladium black for 1 h affording H-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OCH_3 (III), which was then condensed with Z-(im-Bzl)His-OH via the carbodiimide method to give Z-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OCH_3 (IV). Saponification of IV afforded the corresponding acid, Z-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OH (V), which by selective catalytic hydrogenation, as described before, produced the substituted heptapeptide, H-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OH (VI). The latter was ester-

[1] The amino acids are of L-configuration.
[2] A. METALLIDIS and D. THEODOROPOULOS, Chim. Chronika, New series *1*, 154 (1972).
[3] B. RINIKER and R. SCHWYZER, Helv. chim. Acta *47*, 2357 (1964).
[4] The physical data of intermediate peptides and final products are listed in the Table.
[5] H. SCHWARZ and F. M. BUMPUS, J. Am. chem. Soc. *81*, 890 (1959).
[6] D. THEODOROPOULOS and J. GAZOPOULOS, J. chem. Soc. *1960*, 3861.
[7] R. H. MAZUR, Can. J. Chem. *40*, 1098 (1962).
[8] D. THEODOROPOULOS, J. org. Chem. *21*, 1550 (1956). – D. THEODOROPOULOS and G. FÖLSCH, Acta chem. scand. *12*, 1955 (1958).