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Fluorine-18 and Carbon-11 Labeled Amphetamine Analogs—Synthesis, Distribution, Binding Characteristics in Mice and Rats and a PET Study in Monkey

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No-carrier-added (NCA) (±)-*p*-[^{18}F]fluoroamphetamine (**2a**) and (±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine (**2b**) were synthesized through a multistep synthesis by nucleophilic substitution of the appropriate precursors (*p*-nitrobenzaldehyde, **1a** and 6-nitropiperonal **1b**, respectively) with [^{18}F]fluoride followed by condensation with nitroethane and reduction with LAH in 20–30% yield (EOB) in a synthesis time of 90–109 min from EOB. NCA (−)-[^{11}C]methamphetamine (**4a**) and (±)-3,4-methylenedioxy-*N*-[^{11}C]methamphetamine (**4b**) were synthesized by methylation of the appropriate desmethyl precursors **3a** and **3b** with [^{11}C]H_3I in 40–60% yield (EOB) in a synthesis time of 30 min from EOB. Animal studies in mouse and rat revealed that the relative tissue uptake of these radiotracers was kidneys > lungs > liver > spleen > brain > heart > blood. The uptakes of these radiotracers in mouse brain were high and similar at 5 min post-injection (approx. 5%/g) but radioactivity then declined rapidly (approx. 1%/g at 60 min post-injection). For compounds **2a** and **2b**, the activity in the femur did not increase with time indicating *in vivo* defluorination may not be the major route of metabolism. Monoamine uptake inhibitors (nomifensine, fluoxetine and nisoxetine) did not inhibit but enhance the uptake of (−)-[^{11}C]methamphetamine (**4a**) in the rat brain by greater than 50%. A PET study in a Rhesus monkey revealed that the uptakes of (−)-[^{11}C]methamphetamine in different brain regions were similar and the retention of the radioactivity in these regions remained constant throughout the study. Analysis of arterial plasma by HPLC showed that 50% of radioactivity remained as **4a** at 60 min post-injection.

Introduction

The use of illicit drugs in America has devastating effects upon individuals, their families and the country as a whole. Whereas there are numerous drugs being abused in the United States, amphetamine (and its analogs) and cocaine are abused regularly. The physical, mental, emotional and financial damage brought by widespread drug abuse demands basic research into the neurochemical aspects of drug abuse.

(+)-Amphetamine and methamphetamine, like cocaine, are readily self-administered by humans, monkeys and rats (Gritliths *et al.*, 1980; Collins *et al.*, 1984). The immediate effects of these "designer drugs" is to lower reinforcement thresholds for brain stimulation reward (Stein, 1964; Kornetsky and Esposito, 1981). It has been reported that (+)-amphetamine produces euphoria in humans (Jonsson, 1972). The neurobiological mediators of these reinforcing properties appear to be the central catecholamines, most notably the neurotransmitter dopamine.

The binding properties of amphetamine in rat brain are controversial. Paul *et al.* reported that (+)-[^{3}H]amphetamine had specific binding in crude membranes from high (93 nM) and low (300 nM) affinities (Paul *et al.*, 1982a). The specific binding of (+)-[^{3}H]amphetamine was saturable with a dissociation constant (K_d) of 70 nM and a binding site density (B_{max}) of 320 fmol/mg protein (Paul *et al.*, 1982b). Late reports, however, showed that the high

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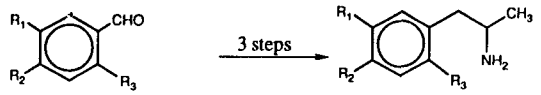


(1a) R_1=R_3=H; R_2= NO_2 (2a) R_1=R_3=H; R_2=^{18}F

(1b) R_1, R_2= OCH_2O; R_3=NO_2 (2b) R_1, R_2= OCH_2O; R_3=^{18}F

Step 1: K[^{18}F]/ Kryptofix 2. 2. 2, DMSO, 140°, 10 min
Step 2: $CH_3CH_2NO_2$, NH_4OAc, $MgSO_4$, 110°, 10 min
Step 3: LAH/ Ether

Scheme 1. Synthesis of (±)-*p*-[^{18}F]fluoroamphetamine and (±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine.

affinity binding site for (+)-amphetamine in rat hypothalamus was an artifact (Lesage *et al.*, 1984) and that (+)-[^{3}H]amphetamine bound to acceptor sites which were not MAO-A but were probably a polar lipid (Lesage *et al.*, 1985). The regional distribution of [^{3}H]amphetamine-binding sites was sensitive to ionic strength of the medium. In the presence of a high- or a low-ionic strength medium, the distribution of binding sites was almost completely reversed (Lesage *et al.*, 1985). Amphetamine is a weak monoamine reuptake inhibitor. It inhibits both noradrenaline and dopamine uptake (Keller *et al.*, 1982). The K_i for dopamine, norepinephrine and serotonin reuptake is 3.6, 0.88 and 8 μM, respectively (Ritz *et al.*, 1987).

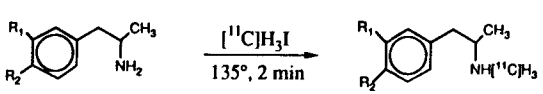
The psychotropic amphetamine analogs, (−)-3,4-methylenedioxyamphetamine (MDA) and (−)-3,4-methylenedioxymethamphetamine (MDMA) displayed moderate affinities for 5-HT_2 sites (K_i = 3.4 and 3.3 μM, respectively) whereas the affinities for their (+)-enantiomers were lower (K_i = 13 and 15.8 μM, respectively). Similar absolute and relative affinities were obtained at 5-HT_1 sites. The binding affinities of MDA and MDMA at D-2 sites were also low (K_i > 25 μM) (Lyon *et al.*, 1986).

In vitro studies also showed that (±)MDA and (±)MDMA had moderate to high affinity (1–10 μM) for 5-HT binding sites in the human cortex (Pierce and Peroutka, 1988) and that [^{3}H](±)MDMA had specific binding sites in the rat brain with a K_d of 99 nM and a B_{max} of 30 fmol/mg protein (Gehlert *et al.*, 1985). A later report, however, indicated that the reported [^{3}H]MDMA binding to rat brain homogenates resulted from an artifactual of [^{3}H]MDMA to glass fiber filter paper (Wang *et al.*, 1987). Thus, [^{3}H]MDMA had extremely limited usefulness in characterizing the potential site or sites of action of MDMA. In order to study the regional distribution and to characterize the *in vivo* binding characteristics of amphetamine and its analogs in the brain, we have synthesized no-carrier-added (NCA) (±)-*p*-[^{18}F]fluoroamphetamine (**2a**), (±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine (**2b**) (Scheme 1), (−)-[^{11}C]methamphetamine (**4a**) and (±)-3,4-methylenedioxy-*N*-[^{11}C]methamphetamine (**4b**) (Scheme 2), studied their distributions in mice, their bindings in the rat brain and performed a preliminary PET study in a monkey. During the course of our studies, a report on the synthesis and biodistribution of [^{11}C]methamphetamine in mice has appeared (Inoue *et al.*, 1990). Preliminary reports of our studies also have appeared (Shiue *et al.*, 1990).

Materials and Methods

Racemic amphetamine, (+)-amphetamine–sulfate, (−)-amphetamine–sulfate, racemic methamphetamine–HCl, (+)-methamphetamine, (−)-methamphetamine, racemic 3,4-methylenedioxyamphetamine–HCl (MDA–HCl), (+)-3,4-methylenedioxyamphetamine–HCl, (−)-3,4-methylenedioxyamphetamine–HCl, racemic 3,4-methylenedioxymethamphetamine–HCl (MDMA–HCl), (+)-3,4-methylenedioxymethamphetamine–HCl and (−)-3,4-methylenedioxymethamphetamine–HCl were either purchased from Sigma Chemical Company, St Louis, Mo. or provided by the National Institute on Drug Abuse. *p*-Nitrobenzaldehyde, 6-nitropiperonal, lithium aluminum hydride (1 M in diethyl ether) were obtained from Aldrich Chemical Company. Fluoxetine and nisoxetine were gifts from Lilly Research Laboratories, Indianapolis, Ind., nomifensine was a gift from Hoechst–Roussel Pharmaceuticals Inc., Somerville, N.J. C_{18} Sep-Pak cartridges were obtained from Waters Chromatography Division, Millipore Corporation. Radioactivity was determined using a calibrated ion chamber (Capintec CRC-12, Capintec, Inc.) and a sodium iodide well counter (Packard, Gamma Counter 5000 Series, Packard Instrument Company, Ill.).

Thin layer chromatographic (TLC) analyses were performed on plastic-backed silica gel TLC plates (Merck). High performance liquid chromatography (HPLC) analyses were carried out with a Sonntek liquid chromatograph equipped with both u.v. and radioactivity monitors. For the preparative separations, a semi-preparative silica gel column (10 × 250 mm Ultremex 5 silica, Phenomenex) was used with CH_3OH:NH_4NO_3 aqueous buffer (98.75:1.25, pH 9.3) as the solvent. The buffer solution was prepared by mixing concentrated ammonium hydroxide (40 mL) and ammonium nitrate (12 g) in water (400 mL) (Smith *et al.*, 1987). For the specific activity determinations, an analytical silica gel column (4.6 × 250 mm Ultremex 5 silica) was used and eluted with the same solvent as that used for the preparative separations and with a flow rate of 1 mL/min.



(3a) R_1=R_2=H (4a) R_1=R_2=H

(3b) R_1, R_2= OCH_2O (4b) R_1, R_2= OCH_2O

Scheme 2. Synthesis of carbon-11 labeled methamphetamine and 3,4-methylenedioxymethamphetamine.

Table 1. Tissue distribution of (±)-*p*-[^{18}F]fluoroamphetamine (**2a**) in mice (mean ± SD)

	Time after injection					
	5 min (n = 2)		30 min (n = 3)		60 min (n = 5)	
Organ	% Dose/organ	% Dose/gram	% Dose/organ	% Dose/gram	% Dose/organ	% Dose/gram
Blood	—	1.95	—	0.81 ± 0.04	—	0.48 ± 0.05
Brain	3.16	5.70	1.29 ± 0.61	2.28 ± 0.49	0.54 ± 0.16	1.43 ± 0.28
Heart	0.70	3.30	0.21 ± 0.07	1.04 ± 0.09	0.70 ± 0.01	0.53 ± 0.09
Lungs	5.13	19.60	1.63 ± 0.84	4.81 ± 0.73	0.92 ± 0.16	6.29 ± 4.41
Liver	12.30	6.90	5.27 ± 2.44	2.93 ± 2.85	5.31 ± 0.53	3.95 ± 0.65
Spleen	0.89	6.16	0.39 ± 0.10	3.29 ± 0.11	0.14 ± 0.04	1.47 ± 0.42
Kidneys	10.3	18.4	5.29 ± 0.52	8.83 ± 3.04	1.67 ± 0.83	4.14 ± 1.36
Small intestines	4.25	5.21	2.46 ± 0.41	2.01 ± 1.50	1.07 ± 0.44	1.38 ± 0.38
Femur	—	1.77	—	0.78 ± 0.10	—	0.88 ± 0.79

No-carrier-added (NCA) (±)-p-[^{18}F]fluoroamphetamine (2a)

NCA compound **2a** was synthesized through a multistep synthesis by nucleophilic substitution of *p*-nitrobenzaldehyde (**1a**) with [^{18}F]fluoride followed by Knoevenagel condensation with nitroethane and reduction with LAH (Scheme 1). Thus, NCA aqueous [^{18}F]fluoride (0.3 mL) prepared by the ^{18}O(p,n)^{18}F reaction in a CTI RDS 112/00 cyclotron on a small volume of enriched water (97% ^{18}O) target was added to a solution of K_2CO_3/Kryptofix 2 · 2 · 2 in a pyrex vessel. The water was evaporated using a stream of nitrogen at 110 °C and coevaporated to dryness with CH_3CN (2 × 0.5 mL). *p*-Nitrobenzaldehyde (9.0 mg in 0.2 mL of DMSO) was added to the dried K[^{18}F] and the solution was kept at 140 °C for 10 min and then cooled to room temperature. Water (3 mL) was added and the solution was passed through a C_{18} Sep-Pak. The C_{18} Sep-Pak was washed with an additional 2 mL of water and the product was eluted with ether (2 × 2 mL). The ethereal solution was filtered through an anhydrous K_2CO_3 column (0.5 × 5 cm), concentrated and nitroethane (300 μL, 310 mg), ammonium acetate (10 mg) and anhydrous magnesium sulfate were added. The mixture was stirred in an oil bath (110 °C) for 10 min. Water (3 mL) was added and the mixture was extracted with ether (2 × 2 mL). The ethereal solution was filtered through another anhydrous K_2CO_3 column (0.5 × 5 cm). Lithium aluminum hydride (0.3 mL,

Table 2. Tissue distribution of (±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine (**2b**) in mice (mean ± SD)

	Time after injection					
	5 min (n = 4)		30 min (n = 4)		60 min (n = 6)	
Organ	% Dose/organ	% Dose/gram	% Dose/organ	% Dose/gram	% Dose/organ	% Dose/gram
Blood	—	5.048 ± 5.04	—	1.33 ± 0.47	—	0.52 ± 0.32
Brain	5.23 ± 1.03	13.02 ± 2.91	1.10 ± 0.32	2.81 ± 1.01	0.47 ± 0.18	1.23 ± 0.64
Heart	0.61 ± 0.12	4.05 ± 1.12	0.23 ± 0.07	1.66 ± 0.50	0.12 ± 0.03	0.66 ± 0.15
Lungs	3.56 ± 0.93	14.73 ± 2.17	1.62 ± 0.63	7.32 ± 2.90	0.73 ± 0.47	2.71 ± 1.70
Liver	12.52 ± 1.73	8.25 ± 1.46	8.34 ± 3.42	6.24 ± 2.86	6.73 ± 1.88	3.83 ± 0.91
Spleen	0.57 ± 0.13	5.41 ± 0.65	0.49 ± 0.22	4.85 ± 2.04	0.28 ± 0.08	2.28 ± 0.95
Kidneys	9.90 ± 0.41	20.95 ± 2.04	4.28 ± 1.60	9.53 ± 3.40	2.10 ± 0.56	3.73 ± 0.71
Small intestines	4.99 ± 1.92	5.84 ± 1.48	2.63 ± 1.70	4.03 ± 1.83	1.61 ± 0.74	1.82 ± 0.88
Femur	—	2.19 ± 1.26	—	1.06 ± 0.35	—	0.50 ± 0.17

Table 3. Tissue distribution of (−)-[^{11}C]methamphetamine (**4a**) in mice (mean ± SD)

	Time after injection					
	5 min (n = 6)		30 min (n = 6)		60 min (n = 4)	
Organ	% Dose/organ	% Dose/gram	% Dose/organ	% Dose/gram	% Dose/organ	% Dose/gram
Blood	—	1.04 ± 0.17	—	0.72 ± 0.10	—	0.38 ± 0.11
Brain	2.21 ± 0.26	5.94 ± 1.31	1.56 ± 0.18	3.68 ± 0.35	0.78 ± 0.15	1.81 ± 0.34
Heart	0.49 ± 0.07	3.45 ± 0.77	0.28 ± 0.05	1.96 ± 0.38	0.13 ± 0.01	0.87 ± 0.12
Lungs	2.66 ± 0.72	14.82 ± 6.14	1.51 ± 0.26	6.22 ± 0.91	0.81 ± 0.07	4.26 ± 0.59
Liver	10.39 ± 2.12	7.91 ± 1.12	10.05 ± 1.66	6.71 ± 0.74	6.10 ± 1.02	4.06 ± 0.53
Spleen	0.62 ± 0.19	7.25 ± 1.51	0.83 ± 0.35	7.95 ± 2.21	0.30 ± 0.05	3.57 ± 0.68
Kidneys	10.91 ± 1.74	27.34 ± 6.57	7.07 ± 2.91	15.77 ± 7.07	2.17 ± 0.06	5.04 ± 0.89
Small intestines	5.21 ± 0.99	6.10 ± 0.93	3.18 ± 0.98	4.15 ± 0.87	2.28 ± 0.41	2.40 ± 0.28

1 M in diethyl ether) was added and the solution was stirred at room temperature for 15 min. Diluted HCl (5%, v/v) was added and the mixture was extracted with ether, dried (K_2CO_3), concentrated to 0.5 mL and injected into semi-preparative HPLC (silica gel, 10 × 250 mm Ultremex 5 silica, Phenomenex; $CH_3OH:NH_4NO_3$ aqueous buffer, 98.75:1.25 as the solvent with a flow rate of 5 mL/min). The effluent of the HPLC column was passed through an u.v. detector at 254 nm and into a radioactivity detector. The fraction containing (±)-*p*-[^{18}F]fluoroamphetamine (**2a**) was collected from 8.4 to 10 min after injection. The HPLC eluate was evaporated, the residue co-evaporated with 1 mL of 2% HCl-ethanol and finally with 5 mL of ethanol to dryness. To the residue was added 3 mL of normal saline and the resulting solution was filtered through a 0.22 μM cellulose acetate membrane filter (Millipore) into a multi-injection vial. The retention time of compound **2a** in the analytical column was 6 min. The radiochemical yield of **2a** was 24–32% (EOB) in a synthesis time of 90 min from EOB. The R_f of compound **2a** was 0.6 [silica gel, $CH_3OH:NH_4OH$ (98:2) as solvent]. Typically, from 87 mCi of aqueous [^{18}F]fluoride, 13 mCi of **2a** was isolated after 90 min from EOB.

*No-carrier-added(±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine(**2b**)*

Compound **2b** was synthesized in a similar manner as that described for the synthesis of compound **2a** except using 6-nitropiperonal (**1b**) as the starting material. The retention time of compound **2b** in the analytical column was 5.2 min. The radiochemical yield of **2b** was 20–25% (EOB) in a synthesis time of 109 min from EOB. The R_f of compound **2b** was 0.57 [silica gel, $CH_3OH:NH_4OH$ (98:2) as solvent].

*No-carrier-added (−)-[^{11}C]methamphetamine (**4a**) and (±)3,4-methylenedioxy-N-[^{11}C]methamphetamine (**4b**)*

NCA compound **4a** was synthesized by methylation of (−)-amphetamine (**3a**) with [^{11}C]H_3I followed by purification with HPLC (Scheme 2). Thus, NCA [^{11}C]H_3I prepared from [^{11}C]O_2 in an automated system (NKK, Japan) was dispensed into a small reaction vessel containing 2 mg of (−)-amphetamine in 300 μL of DMF:DMSO (4:1) at ice-bath temperature. The solution was heated at 135°C for 2 min. The mixture was injected into a semi-preparative HPLC [silica gel, 10 × 250 mm Ultremex 5 silica, Phenomenex; $CH_3OH:NH_4NO_3$ aqueous buffer, (98.75:1.25) as the solvent with a flow rate of 7.5 mL/min]. The effluent of the HPLC column was passed through an u.v. detector at 254 nm and into a radioactivity detector. The fraction containing (−)-[^{11}C]methamphetamine (**4a**) was collected from 9 to 11 min after injection. The HPLC eluate was processed as described above. The radiochemical yield of **4a** was 50–60% (EOB) in a synthesis time of 30 min from EOB with a radiochemical purity of 98%. Thus, from 250 mCi of [^{11}C]O_2, 50 mCi of compound **4a** was obtained 30 min from EOB. The specific activity was 0.2–1.69 Ci/μM (EOB) as determined by the u.v. absorbance of the radioactive peak compared with a standard solution of (−)-methamphetamine. The R_f of compound **4a** was 0.38 [silica gel, $CH_3OH:NH_4NO_3$ aqueous buffer (98.75:1.25) as solvent].

Compound **4b** was synthesized in a similar method except using (±)-3,4-methylenedioxyamphetamine (**3b**) as the starting material. The retention time of **4b** was 11 min in the same HPLC system as compound **4a** with a radiochemical yield of 40–60% (EOB) in a synthesis time of 30 min from EOB. The radiochemical purity of **4b** was 97% with a specific activity of 0.4–1.26 Ci/μM, R_f = 0.44 [silica gel, $CHCl_3:CH_3OH$:Conc NH_4OH (80:20:1) as solvent]. The other enantiomers of compounds **4a** and **4b** were prepared in a similar method.

Table 4. Tissue distribution of (±)3,4-methylenedioxy-*N*-[^{11}C]methamphetamine (**4b**) in mice (mean ± SD)

	Time after injection					
	5 min (n = 4)		30 min (n = 4)		60 min (n = 3)	
Organ	% Dose/organ	% Dose/gram	% Dose/organ	% Dose/gram	% Dose/organ	% Dose/gram
Blood	—	1.02 ± 0.15	—	1.07 ± 0.17	—	0.59 ± 0.12
Brain	1.93 ± 0.38	4.85 ± 0.80	0.82 ± 0.19	1.84 ± 0.38	0.16 ± 0.04	0.39 ± 0.10
Heart	0.37 ± 0.05	2.69 ± 0.43	0.16 ± 0.02	1.03 ± 0.14	0.06 ± 0.02	0.42 ± 0.16
Lungs	2.53 ± 0.79	12.21 ± 3.85	0.65 ± 0.08	3.01 ± 0.34	0.22 ± 0.04	0.99 ± 0.30
Liver	14.03 ± 4.63	9.20 ± 2.97	18.57 ± 2.50	12.55 ± 2.01	11.32 ± 1.76	6.87 ± 0.55
Spleen	0.57 ± 0.25	5.54 ± 1.72	0.37 ± 0.04	2.75 ± 0.70	0.09 ± 0.03	0.80 ± 0.29
Kidneys	8.25 ± 0.44	19.36 ± 1.44	3.44 ± 0.43	7.91 ± 0.97	1.08 ± 0.22	2.44 ± 0.66
Small intestines	4.00 ± 1.33	5.23 ± 0.98	4.49 ± 1.19	4.51 ± 1.51	1.73 ± 0.48	1.61 ± 0.80

Table 5. Distribution of (−)-[^{11}C]methamphetamine in monoamine reuptake inhibitors pretreated rat brain 1 h after injection (mean ± SD) (% dose/gram)

	Pretreatment					
Region	Saline (n = 4)	Fluoxetine* (n = 4)	Saline (n = 4)	Nisoxetine† (n = 4)	Saline (n = 8)	Nomifensine‡ (n = 8)
Frontal cortex	0.25 ± 0.043	0.34 ± 0.032	0.238 ± 0.029	0.245 ± 0.033	—	—
Rest of cortex	0.235 ± 0.049	0.34 ± 0.03	0.23 ± 0.029	0.238 ± 0.034	0.37 ± 0.05	0.635 ± 0.103
Striatum	0.25 ± 0.057	0.333 ± 0.04	0.227 ± 0.022	0.24 ± 0.036	0.355 ± 0.048	0.592 ± 0.082
Cerebellum	0.18 ± 0.039	0.245 ± 0.019	0.175 ± 0.019	0.177 ± 0.021	0.255 ± 0.031	0.452 ± 0.08
Rest of brain	0.197 ± 0.038	0.275 ± 0.024	0.193 ± 0.026	0.203 ± 0.025	0.295 ± 0.048	0.505 ± 0.084
Blood	0.055 ± 0.01	0.058 ± 0.005	0.053 ± 0.017	0.057 ± 0.01	0.07 ± 0.008	0.078 ± 0.017

*Rats were pretreated with 10 mg/kg fluoxetine i.p. 6 h prior to radiotracer injection.
†Rats were pretreated with 10 mg/kg nisoxetine i.p. 1 h prior to radiotracer injection.
‡Rats were pretreated with 6 mg/kg nomifensine i.p. 30 min prior to radiotracer injection.

*Tissue distribution of NCA (±)-p-[^{18}F]fluoroamphetamine (**2a**), (±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine (**2b**), (−)-[^{11}C]methamphetamine (**4a**) and (±)-3,4-methylenedioxy-N-[^{11}C]methamphetamine (**4b**) in mice*

Female CF-1 mice, 25–38 g, were restrained in a plastic holder and injected in a lateral tail vein without anesthesia with 0.3–1.5 mCi of radiotracer (**2a**, **2b**, **4a** or **4b**) in 100 μL of isotonic saline solution. The mice were killed by cervical dislocation and decapitation at 5, 30 and 60 min after injection. The dissected tissues were blotted to remove adhering blood and placed in tared counting vials. A sample of blood was obtained by heart puncture after cervical

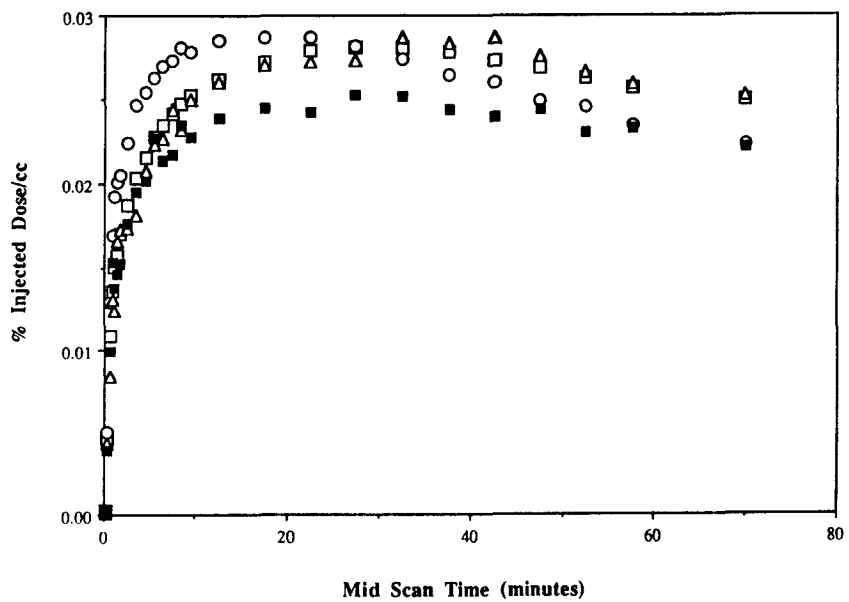


Fig. 1. Time courses for (−)-[^{11}C]methamphetamine distribution to: □, cortex; ○, cerebellum; △, left temporal lobe; and ■, striatum in a Rhesus monkey.

dislocation. The radioactivity in the entire tail was measured in a NaI detector (Packard) to verify the patency of the tail vein injection. If the ratio of percent uptake in the tail over the percent uptake in the muscle was greater than 3, it was considered as a mis-injection. The radioactivity of each sample was also measured in a NaI detector, the sample weighed and the activity expressed as percent of injected dose/organ or percent of injected dose/gram of tissue decay corrected to time of injection.

*Anatomical distribution of NCA (−)-[^{11}C]methamphetamine (**4a**) in rat brain*

Male Sprague–Dawley rats, 290–340 g, were restrained in a plastic holder and injected in a lateral tail vein without anesthesia with 0.2–1 mCi of **4a** in 100 μL of isotonic saline solution. At 1 h after injection, the rats were killed by decapitation. Each brain was rapidly removed, dissected into different regions and placed in tared counting vials. The patency of the tail vein injection was verified as described above. The radioactivity of each sample was measured in a NaI well counter (Packard), the sample weighed and the activity expressed as percent of injected dose/gram of tissue decay corrected to time of injection.

*Effects of monoamine reuptake inhibitors on the biodistribution of (−)-[^{11}C]methamphetamine (**4a**)*

Male Sprague–Dawley rats, 260–360 g, were pretreated i.p. with monoamine reuptake inhibitors (fluoxetine, 10 mg/kg, nisoxetine, 10 mg/kg and nomifensine, 6 mg/kg, respectively) 30 min to 6 h prior to lateral tail vein injection of compound **4a** (0.2–2 mCi in 0.1 mL of isotonic saline solution). At 1 h after injection, the rats were killed by decapitation. Each brain was rapidly removed, dissected into different regions and placed in tared counting vials. A sample of blood was obtained by heart puncture after decapitation. The patency of the tail vein injection was verified as described above. The radioactivity of each sample was measured in a NaI well counter, the sample weighed and the activity expressed as percent of injected dose/gram of tissue decay corrected to time of injection.

PET study of (−)-[^{11}C]methamphetamine in a monkey

A male Rhesus monkey (*Macaca mulatta*) weighing 12 kg was initially anesthetized with 17 mg/kg of ketamine administered i.m. approx. 2.5 h prior to isotope injection. Once the monkey was anesthetized pentobarbital (20 mg/kg) was administered i.v. followed by insertion of an endotracheal tube. The monkey was maintained under pentobarbital anesthesia for the remainder of the study. The monkey had previously been used for long-term cardiovascular monitoring and had indwelling arterial and venous catheters. These catheters were used to administer the radioligand and to obtain blood samples. After the study, the monkey was returned to its home cage and allowed to fully recover from anesthesia.

The positron emission tomography used for these studies was a Computer Technology and Imaging (CTI/Siemens) whole body ECAT 931/08-16 PET scanner. The monkey's head was placed in a plastic head holder and a foam mold (Alpha Cradle Imaging Head Immobilizer, Smithers Medical Products, Inc., Tallmadge, Ohio) was used to stabilize the head. Before the radioligand injection, a 20 min transmission scan was performed using eight ^{68}Ge filled ring sources to measure regional attenuation. The monkey was injected (i.v. bolus) with 32.5 mCi of (−)-[^{11}C]methamphetamine (**4a**). The PET scans were acquired continually over an 80 min period. Six 15 s, one 30 s, eight 1 min, ten 5 min and one 20 min scan were taken in the dynamic mode. Regions of interest corresponding to cortex, cerebellum, temporal lobe and striatum were selected by co-registration of PET/MRI images. The MR images (Siemens, 1.0 Tesla 42 SP Magnetic Resonance Imager) were taken in overlapping 4 mm thick slices at 2 mm intervals over the entire monkey brain. Four contiguous 4 mm thick MR slices taken at 2 mm intervals will therefore correspond to the volume covered in a single 8 mm thick PET slice. MR images were converted to PET format and displayed using available Siemens software (Imagetool™) side-by-side with corresponding PET images. Because the same rigid custom head immobilization mold used for the PET scan was also used with the monkey on the MRI scanner, identical orientations and axial positioning between the PET and MR images were ensured. Regions identified on the MR images were manually drawn using Imagetool™ and transferred to the corresponding PET scans. The cortex, cerebellum and temporal lobe are sufficiently large structures in the monkey brain that quantitative assessment of PET radioligand concentrations of these structures are only minimally affected by partial volume effects.

Monkey plasma metabolite analysis

After injection of (−)-[^{11}C]methamphetamine, blood samples were collected from the previously mentioned indwelling arterial catheters. During the first 2 min, 1 mL blood samples were drawn manually at approx. 10 s intervals. More blood samples were collected at 5, 10, 30, 60 and 80 min post-injection. Blood samples were placed in heparinized tubes and centrifuged. Duplicate aliquots (0.3 mL) of plasma sample were counted in the gamma counter and the counts corrected for background and decay. Selected plasma samples (0, 1, 5, 30 and 60 min) were analyzed for radioactive metabolite(s) and unchanged (−)-[^{11}C]methamphetamine. An aliquot of plasma (0.2–0.4 mL) was added to 2 mL of CH_3OH, vortexed and centrifuged. The supernatant, with the addition of an authentic (−)-methamphetamine (5 μg in 10 μL H_2O) was injected into the HPLC system (4.6 × 250 mm Ultremex 5 silica column, Phenomenex; CH_3OH:NH_4NO_3 aqueous buffer, 98.75:1.25, 1.8 mL/min). The retention time of (−)-methamphetamine was 9.5 min in this system.

Results and Discussion

Widespread abuse of amphetamine and its analogs has prompted us to label these drugs with positron-emitters with doses below the pharmacological activity threshold such that elucidation of metabolic pathways and mechanism of action can be carried out. A few positron-emitter labeled amphetamines have been synthesized, such as [^{13}N]amphetamine (Finn *et al.*, 1981), [^{11}C]amphetamine (Schoeps *et al.*, 1991) and [^{11}C]methamphetamine (Inoue *et al.*, 1990). In addition, several bromine and iodine labeled amphetamine analogs also have been synthesized (Sargent *et al.*, 1975, 1984; Coenen, 1981). We have synthesized four ^{18}F- or ^{11}C-labeled amphetamine and their analogs, studied their distribution and *in vivo* binding characteristics in mice and rats and performed a preliminary PET study in a monkey.

No-carrier-added (±)-*p*-[^{18}F]fluoroamphetamine (**2a**) was synthesized through a multistep synthesis by nucleophilic substitution of *p*-nitrobenzaldehyde (**1a**) with [^{18}F]fluoride followed by Knoevenagel condensation with nitroethane and reduction with LAH in 24–32% yield in a synthesis time of 90 min from EOB (Scheme 1). Attempt to synthesize compound **2a** by nucleophilic substitution of 1-(*p*-nitrophenyl)-2-nitropropene with [^{18}F]fluoride followed by reduction with LAH gave **2a** in poor yield. NCA (±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine (**2b**) was synthesized in a similar manner. Nucleophilic substitution of 6-nitropiperonal (**1b**) with [^{18}F]fluoride gave 6-[^{18}F]fluoropiperonal (Ding *et al.*, 1990) in 55% yield. Condensation of 6-[^{18}F]fluoropiperonal with nitroethane followed by reduction with LAH gave compound **2b** in 20–25% yield in a synthesis time of 109 min from EOB.

Carbon-11 labeled (−)-methamphetamine (**4a**) and (±)-3,4-methylenedioxymethamphetamine (**4b**) were synthesized by methylation of the appropriate desmethyl precursor with [^{11}C]H_3I at 135°C for 2 min followed by purification with HPLC in 40–60% yield in a synthesis time of 30 min from EOB (Scheme 2).

Tables 1–4 show that the relative tissue uptake of these radiotracers in mouse after i.v. administration is kidneys > lungs > liver > spleen > brain > heart > blood which is in agreement with the results of Fuller and Hines (1967). The uptakes of these radiotracers in mouse brain are high and similar at 5 min post-injection. However, the rate of the clearance of these radiotracers from mouse brain is different, with compound **4b** being the fastest. During the course of our studies, the synthesis and distribution of (+)- and (−)-[^{11}C]methamphetamine in mice were reported (Inoue *et al.*, 1990). Our results are in agreement with the reported values. For (±)-*p*-[^{18}F]fluoroamphetamine and (±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine, the activity in the femur did not increase with time indicating *in vivo* defluorination may not be the major route of metabolism.

The neurotoxicities and reinforcing properties of amphetamine and its analogs appear to be due to the release and inhibition of the reuptake of the central catecholamines. Nomifensine, fluoxetine and nisoxetine are the high affinity and high selective inhibitors of dopamine, serotonin and norepinephrine reuptake systems with IC_{50} of 150, 25 and 1–3 nM, respectively (Hunt *et al.*, 1974; Wong *et al.*, 1975; Wong and Bymaster, 1976). In order to study the *in vivo* binding characteristics of amphetamine and its analogs in the rat brain, rats were pretreated with nomifensine, fluoxetine and nisoxetine, respectively. Table 5 shows that (−)-[^{11}C]methamphetamine was distributed quite uniformly in five brain areas and the activity in one brain area never exceeded the activity in other areas by more than a factor of 2. Pretreatment of rats with nomifensine, fluoxetine and nisoxetine did not alter significantly the patterns of the distribution of (−)-[^{11}C]methamphetamine in the rat brain and the uptake in blood, but it did enhance the whole brain uptake. The exact reason(s) for these effects is unclear. Desipramine and other tricyclic antidepressant agents have been shown to increase the level of brain amphetamine presumably by a blockade of the hydroxylation of amphetamine (Dolfini *et al.*, 1969; Lew *et al.*, 1971). The enhancement of (−)-[^{11}C]methamphetamine brain uptake by these three monoamine reuptake inhibitors may be also due to the inhibition of the metabolism of (−)-[^{11}C]methamphetamine *in vivo* by these three monoamine reuptake inhibitors.

The appearance of metabolites in the monkey plasma following injection of compound **4a** was relatively slow. Analysis of arterial plasma by HPLC showed that 50% of radioactivity in the plasma remained as parent compound **4a** at 60 min post-injection.

The distribution of radioactivity in a monkey brain following injection of (−)-[^{11}C]methamphetamine is depicted in Fig. 1. Unlike the uptake and retention in mouse brain, the uptakes of radioactivity in different monkey brain regions (striatum, cerebellum, cortex and left temporal lobe) were similar and the retention of the radioactivity in these regions remained constant throughout the study (80 min). This is probably due to species differences. The neurochemical and neurodegenerative effects of MDMA also have been shown to be species dependent (DeSouza *et al.*, 1990). The fact that (−)-[^{11}C]methamphetamine has a long biological half-life in monkey brain is coincidental with the time course of subjective symptoms. The PET studies of other amphetamine analogs (MDA, MDMA and their enantiomers) in monkeys are continuing.

Conclusion

NCA (±)-*p*-[^{18}F]fluoroamphetamine (**2a**) and (±)-6-[^{18}F]fluoro-3,4-methylenedioxyamphetamine (**2b**) were synthesized in 20–30% yield (EOB) in a synthesis time of 90–109 min from EOB. NCA (−)-[^{11}C]methamphetamine (**4a**) and (±)-3,4-methylenedioxy-*N*-[^{11}C]methamphetamine (**4b**) were synthesized in 40–60% yield in a synthesis time of 30 min from EOB. Animal studies in mouse and rat revealed that the relative tissue uptake of these radiotracers was kidneys > lungs > liver > spleen > brain > heart > blood. The uptakes of these radiotracers in mouse brain were high and similar at 5 min post-injection but radioactivity then declined rapidly. For compounds **2a** and **2b**, the activity in the femur did not increase with time indicating *in vivo* defluorination may not be the major route of metabolism. Monoamine uptake inhibitors (nomifensine, fluoxetine and nisoxetine) did not inhibit but enhance the uptake of (−)-[^{11}C]methamphetamine in the rat brain. A PET study in a Rhesus monkey revealed that the uptakes of (−)-[^{11}C]methamphetamine in different brain regions were similar and the retention of the radioactivity in these regions remained constant throughout the study. Analysis of arterial plasma by HPLC showed that 50% of radioactivity in the plasma remained as **4a** at 60 min post-injection.

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