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Use of Positron Emission Tomography to Study the Dynamics of Psychostimulant-Induced Dopamine Release

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MACH, R. H., M. A. NADER, R. L. E. EHRENKAUFER, S. W. LINE, C. R. SMITH, H. D. GAGE AND T. E. MORTON. *Use of positron emission tomography to study the dynamics of psychostimulant-induced dopamine release.* PHARMACOL BIOCHEM BEHAV **57**(3) 477–486, 1997.—Microdialysis studies have shown that psychostimulants act through a common neurochemical mechanism of elevating synaptic dopamine content in the mesocorticolimbic dopaminergic system. However, little information is available regarding the dynamics of the interaction between the elevated synaptic dopamine levels induced by a psychostimulant and postsynaptic dopamine receptors. The goal of the current investigation was to determine if positron emission tomography (PET) studies using the dopamine D_2-selective radioligand [^{18}F]4′-fluoroclebopride ([^{18}F]FCP) could be used to measure synaptic dopamine levels. Rhesus monkeys were used because our previous studies revealed that [^{18}F]FCP has a low test/retest variability in this species. Under control conditions, [^{18}F]FCP had a high uptake and slow rate of washout from the basal ganglia, a region of brain that expresses a high density of D_2 receptors, reaching kinetic equilibrium at ~40 min. Challenge studies, each separated by at least 1 month, were conducted by administering an intravenous dose of (−)cocaine, *d*-amphetamine, methylphenidate, or *d*-methamphetamine (1.0 mg/kg) at 40 min post-IV injection of a no-carrier-added dose of [^{18}F]FCP. In each case, the psychostimulant caused an increase in the rate of washout of [^{18}F]FCP from the basal ganglia. Methamphetamine and amphetamine had more pronounced effects on the washout kinetics of [^{18}F]FCP relative to cocaine and methylphenidate, a result that is consistent with the ability of each drug to elevate synaptic dopamine levels. Our results indicate that challenge studies with [^{18}F]FCP may be a useful technique for studying the dynamics of the interaction between psychostimulant-induced increases in synaptic dopamine and postsynaptic D_2 receptors.

Positron emission tomography Dopamine D_2 receptors Psychostimulants Rhesus monkey

PSYCHOSTIMULANTS represent a structurally diverse class of compounds, many of which have high abuse liability in humans. Examples of psychostimulants that have high abuse potential are cocaine, the principal alkaloid found in the shrub *Erythroxylon coca*, and the phenethylamines *d*-amphetamine and *d*-methamphetamine. When given to laboratory animals, cocaine, amphetamine, and methamphetamine produce a number of behavioral characteristics such as increased locomotor activity and stereotypies, such as head bobbing and rearing, especially when given in high doses (13,18,30). However, the behavioral characteristic of central nervous system (CNS) stimulants that is primarily responsible for their high abuse potential is their positive reinforcing effects, as evidenced by their ability to maintain drug self-administration in both humans and laboratory animals (11,19,44,54).

Although amphetamine, methamphetamine, and cocaine are known to interact with a number of neurotransmitters in the brain, including serotonin and norepinephrine, the positive reinforcing effects of these drugs are believed to be related to their effects on CNS dopaminergic function (20). Self-administration studies in monkeys have shown that the *d*-stereoisomer of amphetamine and methamphetamine is behaviorally more potent than the corresponding *l*-stereoisomer (1). The observation that *d*-amphetamine is more potent than *l*-amphetamine in inhibiting dopamine uptake, whereas the *d*-and *l*-isomers are equipotent in inhibiting norepinephrine

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uptake, suggests that the reinforcing effects of *d*-amphetamine are mediated through the dopaminergic system (21). There is also a strong correlation between the reinforcing potency of cocaine-like drugs and their binding affinity for the membrane-bound dopamine transporter site; no such relationship exists with the serotonin and norepinephrine transporter sites (22,38). Microdialysis studies have also revealed that psychostimulants, as well as other abused substances such as ethanol and opiates, display the common mechanism of elevating synaptic dopamine content in regions of the brain that receive input from the mesocorticolimbic dopaminergic system (9,15). Amphetamine has been shown to elevate synaptic dopamine levels by blocking the membrane-bound dopamine transporter as well as promoting release from the intracellular pool, whereas the effects of cocaine are solely mediated through the blockade of the dopamine transporter (37). The observations described above have led to the general acceptance that the positive reinforcing effects of psychostimulants are mediated through their ability to indirectly activate the mesocorticolimbic dopaminergic system.

The studies above clearly suggest that the primary site of action of psychostimulants occurs at the presynaptic dopaminergic terminal. However, behavioral studies indicate that it is the interaction of dopamine with postsynaptic receptors that is responsible for the reinforcing effects of these drugs. For example, lesioning of the mesocorticolimbic dopaminergic neurons of the ventral tegmental area (VTA) and nucleus accumbens (NAc) results in extinction of cocaine and amphetamine self-administration (20). A great deal of information regarding the neuropharmacological mechanisms of psychostimulant reinforcement has also been obtained from self-administration studies using selective dopamine receptor antagonists. Presession administration of either a selective D_1 or D_2 antagonist has been shown to antagonize the reinforcing effects of psychostimulants, resulting in rightward shifts in the self-administration dose–response curves (2). Substitution studies, in which a test compound is substituted for the self-administered psychostimulant, have reported that agonists at D_1 (41,50,51), D_2 (53), and D_3 (3,34) receptors can function as reinforcers, indirectly implicating these postsynaptic receptor subtypes in psychostimulant reinforcement.

Although microdialysis studies have been essential in identifying the ability of psychostimulants to elevate synaptic dopamine levels, this procedure does not provide information regarding the functional consequences of this effect, i.e., the interaction of synaptic dopamine with postsynaptic dopamine receptors. One method that has proven to be useful in this regard is positron emission tomography (PET). PET is a functional imaging technique that can measure the regional and temporal uptake and clearance rates of a positron-emitting radiotracer in the living brain. This procedure has been used traditionally to measure differences in receptor density (i.e., receptor binding potential) between control and experimental groups. However, it is now apparent that PET studies using radiotracers that possess a low nanomolar receptor affinity and reversible tissue pharmacokinetics can be used to measure differences in receptor availability caused by changes in synaptic neurotransmitter concentration. For example, PET imaging studies with [^{11}C]raclopride have revealed a dramatic reduction in D_2 receptor binding potential, relative to baseline studies, when an agent that elevates synaptic dopamine is administered immediately prior to a no-carrier-added dose of the radiotracer (7,48). Similar reductions in D_2 receptor binding potential have also been demonstrated using drugs such as anticholinergics or serotonin 5-HT_2 antagonists that are known to disinhibit striatal dopamine release (6,7). Furthermore, *increases* in D_2 receptor binding potential have been observed with [^{11}C]raclopride under conditions of drug-induced potentiation of GABAergic transmission, a result that is consistent with an increase in D_2 receptor availability due to prolonged inhibition of the nigrostriatal dopaminergic system (8). These results clearly indicate that PET studies with a radiotracer such as [^{11}C]raclopride can provide valuable information regarding synaptic dopamine content by measuring differences in receptor availability under conditions known to enhance or reduce dopamine release rates.

We recently reported the development of a new fluorine-18 labeled benzamide derivative, [^{18}F]4′-fluoroclebopride ([^{18}F]FCP) (Fig. 1), that binds reversibly to D_2 receptors under the conditions of PET (23,27). The in vitro binding properties of FCP are also similar to raclopride because both compounds have approximately an equal affinity for dopamine D_2 and D_3 receptors and a relatively low binding affinity for dopamine D_4 receptors (Table 1). Like raclopride, FCP is an antagonist at dopamine D_2-like receptors. These data suggest that [^{18}F]FCP should behave in a manner analogous to that of [^{11}C]raclopride with respect to measuring changes in D_2 receptor binding potential under conditions of elevated or reduced synaptic dopamine levels. Furthermore, it should be possible to conduct PET imaging studies under conditions of pharmacological challenge that are difficult, if not impossible, to conduct with [^{11}C]raclopride because of the shorter half-life of carbon-11 ($t_{1/2}$ = 20.4 min) vs. that of fluorine-18 ($t_{1/2}$ = 109.8 min). For example, PET studies of [^{18}F]FCP in rhesus monkeys revealed that there is peak uptake of radioactivity in the basal ganglia, a region of the brain with a high expression of D_2 receptors, at 40 min post-IV injection of a no-carrier-added dose of the radiotracer. This point of peak accumulation is followed by a phase displaying a linear rate of radiotracer washout that persists for the remainder of the PET imaging study (140 min). Therefore, the administration of a drug that increases synaptic dopamine content at the initial stage of this washout phase could provide information regarding the magnitude and duration (i.e., dynamics) of dopamine release induced by the drug. The goal of these preliminary experiments was to determine if PET imaging studies of this type can be used to examine the dynamics of dopamine release induced by various psychostimulants. These studies could provide valuable information regarding one of the functional consequences of psychostimulants, the interaction of psychostimulant-induced dopamine release with postsynaptic D_2 receptors.

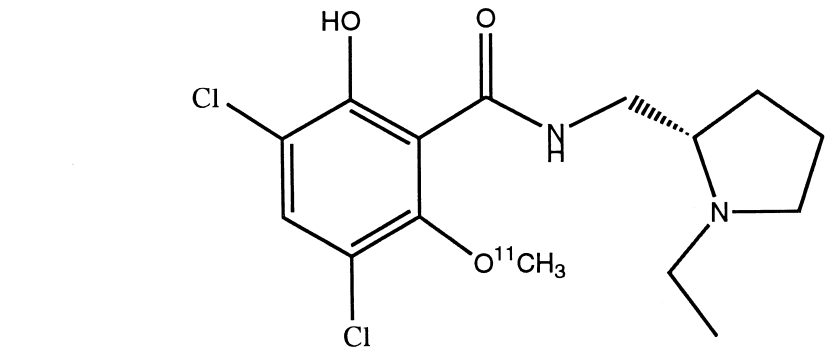
METHODS

Radiosynthesis

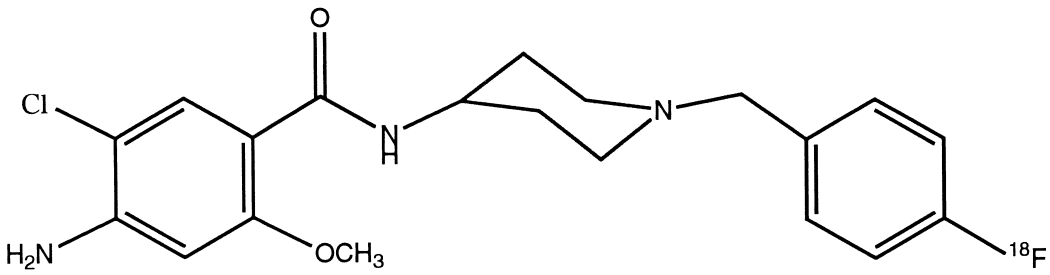
The synthesis of [^{18}F]FCP was accomplished via *N*-alkylation of the corresponding *des*-benzyl precursor with [^{18}F]4-fluorobenzyliodide ([^{18}F]FBI), as described previously (24,26). The synthesis time was ~120 min, and the overall yield ranged from 10 to 56%; the specific activity of the final product ranged from 1000 to 5000 mCi/μmol.

PET Data Acquisition in Rhesus Monkeys

Images were acquired on a Siemens CTI 951/31 PET Scanner. The in-plane resolution of the scanner was measured to be 6.0–6.5 mm full width at half maximum (FWHM) in the dynamic mode and 5.2 mm in the wobble mode. The measured axial resolution was 4.7 mm and 5.5 mm at the axis and 10 cm



[^{11}C]Raclopride



[^{18}F]FCP

FIG. 1. Structures of [^{11}C]raclopride and [^{18}F]FCP.

from the axis of the scanner, respectively. Twenty-six frames were acquired over the 3-h scan period, with the following frame sequences: 5 × 1 min, 5 × 2 min, 5 × 5 min, 8 × 10 min, and 3 × 20 min. Regions of interest were drawn over the basal ganglia (BG) and cerebellum (Cb). Tissue–time activity curves were constructed by plotting the percent injected dose per cm^3 tissue (% I.D./cm^3). The basal ganglia:cerebellum (BG:Cb) ratio was calculated by dividing the % I.D./cm^3 of the basal ganglia by the same value for the cerebellum.

PET imaging studies were carried out in a single adult male rhesus monkey (*Macaca mulatta*; ~10 kg) in order to minimize the experimental error due to between-subject differences. Prior to the start of a PET study, the monkey was initially anesthetized with an intramuscular dose of ketamine (10 mg/kg). The animal was intubated with a 5.0 trachea tube and anesthesia was maintained throughout the entire procedure with 1.5–2.0% isoflurane (Stephens Anesthetic Machine, Artarmon, New South Wales, Australia). The femoral artery was isolated and a 20-ga intravenous catheter was inserted and secured to the artery wall with 3.0 PDS II suture. A 20-ga intravenous catheter was also inserted into the saphenous vein of the opposite leg for administration of the radiotracer. Following implantation of the catheters, the subject was positioned in the PET scanner and a 15-min transmission scan was conducted. The temperature of the monkey was maintained through the use of a heating pad (temperature of the circulating water = 40°C) during the entire transmission and emission scanning procedure. The following vital signs were also constantly monitored throughout the scanning procedure: heart rate, blood pressure, respiration rate, oxygen saturation, and body temperature. In addition, lactated Ringer's was administered intravenously at a rate of 5 ml/kg/h for fluid maintenance.

For the baseline D_2 imaging studies (test/retest studies), the subject was given a bolus injection of radiotracer (3.6–4.4 mCi) and arterial blood samples were collected into preheparinized tubes for analysis. The first 50 samples (0–125 s post-IV injection) were taken using an Ole Dich Instruments (Denmark) automatic blood sampler. Each sample was 0.3 ml, and samples were taken at intervals of 2.5 s. Remaining samples (3, 5, 7.5, 10, 15, 20, 30, 60, 90, 120, and 180 min) were taken manually and were 0.5–1.5 ml in volume. Samples were centrifuged and plasma aliquots of 50–100 μl were counted in a calibrated Packard Cobra II Auto-Gamma Counter (Meriden, CT, USA).

TABLE 1

IN VITRO BINDING PROPERTIES OF RACLOPRIDE AND FCP FOR DOPAMINE D_2, D_3, AND D_4 RECEPTORS

Compound	D_2-like	$D_{2(long)}$	D_3	D_4
FCP[a]	0.95 ± 0.22	5.70 ± 1.52	5.46 ± 0.62	144 ± 21
Raclopride	2.08 ± 0.32[b]	1.8 ± 0.1[c]	3.5 ± 0.3[c]	237[d]

References: [a](27), [b](12), [c](43), [d](45).

For the challenge studies, the subject was given an IV injection (1.0 mg/kg) of (−)cocaine, *d*-amphetamine, methylphenidate, or *d*-methamphetamine at 40 min post-IV injection of a no-carrier-added dose of [^{18}F]FCP. The injection volume for the psychostimulants was 1.0 ml/10 kg. PET data acquisition and arterial blood sampling continued as described above for the baseline studies. Each PET study was conducted on a separate day, and the time interval between PET studies ranged from 6 weeks to 10 months. Acute administration of amphetamine and cocaine has been reported to not alter dopaminergic terminal activity in vivo (14,52). Therefore, it is unlikely that acute doses of amphetamine or cocaine interfered with the subsequent challenge experiments.

Metabolite Analysis of Rhesus Arterial Blood Samples

The correction of arterial blood curves for the presence of metabolites was accomplished using a solid-phase extraction (SPE)–HPLC procedure (10). Arterial blood samples (0.3–1.5 ml) were collected at 1, 10, 20, 60, and 120 min post-IV injection and centrifuged in heparinized microcentrifuge tubes and the plasma was recovered. A plasma sample (400 μl) was added to a mixture of methanol (2 ml) and 0.4 M perchloric acid (4 ml) and the suspension was sonicated with a Vibra Cell microsonication probe (Sonics & Materials Inc., Danbury, CT, USA). The suspension was centrifuged and the supernatant was decanted into a 10-ml syringe containing 4 ml of water. The mixture was applied to an activated C-18 Sep-Pak (Millipore Corporation, Milford, MA, USA). The Sep-Pak was washed with water (3 ml), 1 N sodium hydroxide (2 ml), water (1 × 2 ml, 2 × 1 ml), and methanol (5 ml). Washes were counted in a Packard Cobra II Auto-Gamma Counter. The methanol wash was concentrated in vacuo, rediluted in methanol (300 μl), and filtered. A sample (100 μl) was then chromatographed by C-18 reversed-phase column HPLC (methanol/0.1 M ammonium formate, 4:1). Samples were collected at 1-min intervals for 15 min and counted. The percentage of unchanged FCP and its metabolites was calculated by dividing the amount of recovered radioactivity in the peak by the sum of the total recovered radioactivity in all samples and multiplying by 100.

PET Data Analysis of Baseline and Challenge Studies

The data from test/retest and the challenge studies were normalized by dividing the % I.D./cm^3 at each time point by the value observed in frame 15 (midpoint value is 37.5 min post-IV injection of [^{18}F]FCP). This normalization was necessary to correct for the shift in the peak uptake of radioactivity in both the BG and Cb observed in each study. This shift in peak uptake value of [^{18}F]FCP was due to minor differences

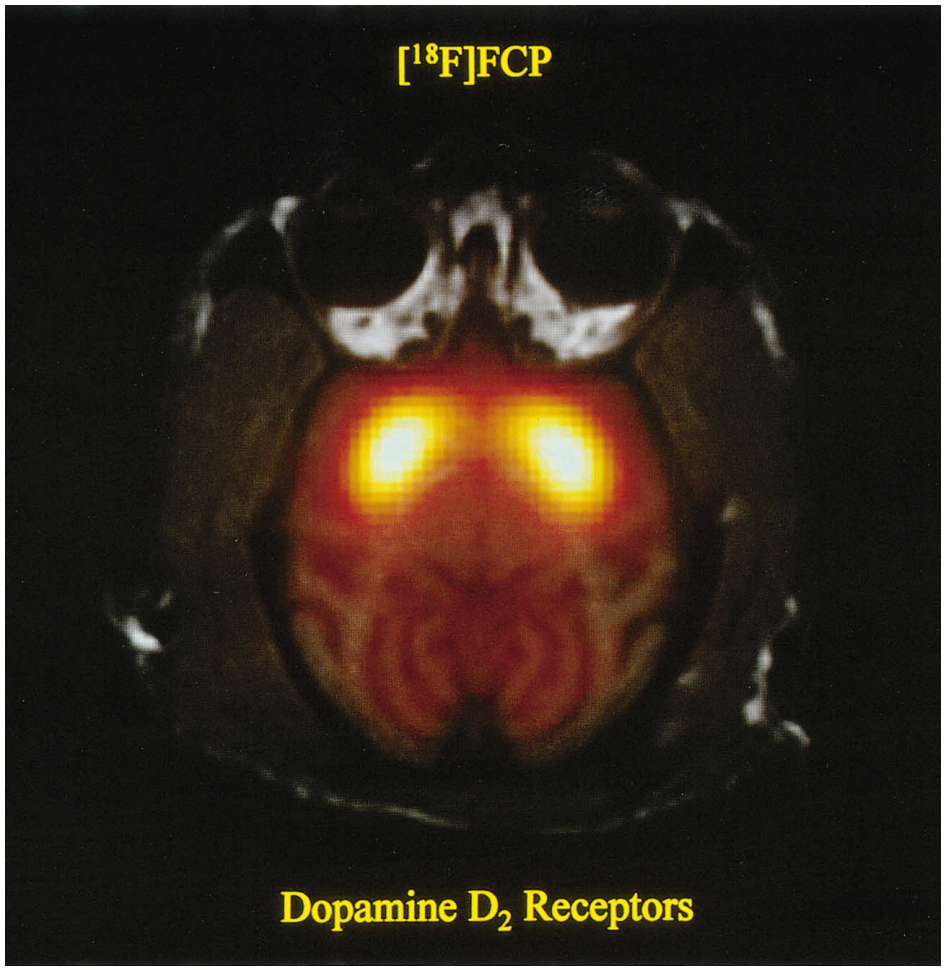


FIG. 2. Transaxial slice of a PET imaging study in a rhesus monkey coregistered with MRI.

in global cerebral perfusion caused by the anesthetic (isoflurane). The change in uptake of [^{18}F]FCP in the basal ganglia (BG) for the challenge studies relative to the baseline (test/retest) studies is defined as:

$$\Delta Y = (\text{normalized uptake in BG})_{\text{challenge}} - (\text{normalized uptake in BG})_{\text{baseline}}$$

A plot of $-\Delta Y$ vs. time (t) is linear after the time of challenge (40 min) and eventually reaches a constant value. When $-\Delta Y$ = constant, $(\text{rate of washout})_{\text{challenge}} = (\text{rate of washout})_{\text{baseline}}$ and indicates the point in time in which the effect of the psychostimulant upon dopamine release has subsided.

RESULTS

The results of a PET imaging study, coregistered with an MRI image of the test subject, are shown in Fig. 2. Note the high uptake of [^{18}F]FCP in the basal ganglia, a brain region expressing a high density of dopamine D_2 receptors. Representative tissue–time activity curves from a dynamic imaging study are shown in Fig. 3. There is a high accumulation of radiotracer in the BG (Fig. 3, open triangles) and a low uptake and rapid rate of washout of radioactivity from the Cb, a reference region possessing a low density of D_2 receptors (Fig. 3, circles). The BG–Cb curve (Fig. 3, closed triangles) is an approximation of the specific binding of [^{18}F]FCP to D_2 receptors. The uptake and specific binding of [^{18}F]FCP reached its peak value at 40 min post-IV injection and was followed by a linear rate of washout that lasted for the duration of the PET data acquisition period. This feature of [^{18}F]FCP (i.e., peak accumulation at 40 min followed by a linear rate of tracer washout) has been observed in every baseline study conducted in rhesus monkeys to date (n = 12). The linear rate of tracer washout provided a stable baseline for studying the effects of an agent that elevates synaptic dopamine levels. Agents that elevate dopamine levels are expected to result in an increase in the rate of [^{18}F]FCP washout due to increased competition between radiotracer and neurotransmitter for D_2 receptors. This effect should persist until levels of synaptic dopamine return to baseline or near-baseline levels.

The results of baseline PET studies and challenge studies using four psychostimulants are shown in Fig. 4. In each case, the intravenous injection of the psychostimulant (1.0 mg/kg) at 40 min post-IV injection of [^{18}F]FCP resulted in a dramatic increase in the rate of washout of the radiotracer from the BG. As shown in Fig. 4, *d*-methamphetamine resulted in the largest reduction in [^{18}F]FCP binding, followed by *d*-amphetamine, cocaine, and methylphenidate.

The effect of the psychostimulant on [^{18}F]FCP washout from the BG was also evaluated by constructing a plot of the difference in the normalized uptake of the radiotracer in the BG under conditions of challenge and the baseline scans (termed $-\Delta Y$) vs. time (Fig. 5). The plot of $-\Delta Y$ vs. time showed a linear increase beginning at the time of challenge that continued until a plateau phase was reached. This plateau phase represents the point in time at which the rate of [^{18}F]FCP washout under conditions of the psychostimulant challenge is equal to the rate of washout under control conditions. This plateau phase identifies when the effect of the psychostimulant-induced increase in synaptic dopamine levels has subsided. In each challenge experiment, the plateau phase was reached at ~100 min post-IV injection of [^{18}F]FCP. These data indicate that the time course for the competition be-

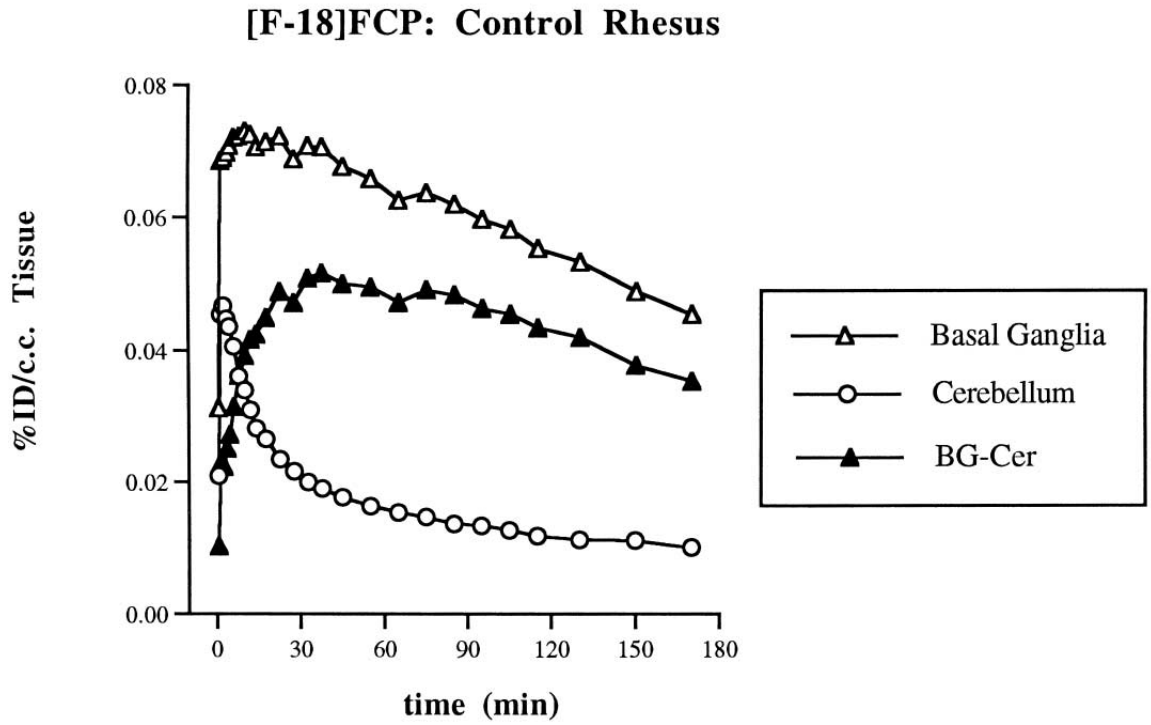


FIG. 3. A representative tissue–time activity curve from a baseline PET study with [^{18}F]FCP in a rhesus monkey. The BG–Cb (BG–Cer in figure) curve is an approximation of the specific binding of [^{18}F]FCP to D_2 receptors.

tween the elevated synaptic dopamine levels and [^{18}F]FCP for D_2 receptors was approximately the same for each psychostimulant. However, the rate of increased radiotracer washout from the BG, as indicated by the slope of the linear region of Fig. 5, was greater for methamphetamine and amphetamine relative to cocaine and methylphenidate. The values of $-\Delta Y$ at the plateau phase also had the following rank order: methamphetamine > amphetamine > cocaine ~ methylphenidate. The difference in the value of $-\Delta Y$ cannot be attributed to differences in the metabolite-corrected arterial blood curve (i.e., the input function) (data not shown) and is believed to reflect the increase in competition of synaptic dopamine with [^{18}F]FCP for D_2 receptor sites under conditions of psychostimulant challenge.

DISCUSSION

It is now generally accepted that the positive reinforcing effects of psychostimulants are mediated, in large part, through the elevation of synaptic dopamine concentrations in the mesocorticolimbic dopaminergic system. Although the principal site of action for many psychostimulants resides on molecular targets located on the presynaptic dopaminergic terminal, the reinforcing effects of psychostimulants are mediated, in large part, through the interaction of the elevated synaptic dopamine with postsynaptic D_1, D_2, and D_3 receptors (2,3,29,34, 41,51). Therefore, methods that could be used to study the interaction between synaptic dopamine and postsynaptic dopamine receptors could potentially provide valuable information about differences in the mechanism of action of drugs of abuse.

Previous PET studies with the radiotracer [^{11}C]raclopride, an agent that binds reversibly to dopamine D_2 receptors, suggested that this functional imaging procedure is a promising tool for studying the effects of changing dopamine levels on D_2 receptor availability. A dramatic decrease in D_2 availability, as measured by reduction in the in vivo binding potential of [^{11}C]raclopride, has been reported in several PET studies in which an IV injection of a drug known to elevate synaptic dopamine levels was administered immediately prior to a no-carrier-added injection of radiotracer (7,48).

The goal of the current study was to determine if PET studies with [^{18}F]FCP could be used to study the dynamics of dopamine release induced by psychostimulants that are known to elevate synaptic dopamine levels through presynaptic mechanisms. The current study takes advantage of a number of properties of [^{18}F]FCP that could be used to assess both the magnitude and duration of the interaction of synaptic dopamine levels and postsynaptic D_2 receptors. These properties include: a) a high affinity and selectivity for dopamine D_2 receptors vs. D_1 receptors and nondopaminergic receptors, indicating that the data acquired in PET reflect the binding of radiotracer to D_2 receptors (25); b) reversible in vivo binding kinetics indicating that the binding of radiotracer to D_2 receptors will be influenced by synaptic dopamine levels; c) BG:Cb ratios of ~5.0 in rhesus monkeys, which provide a high signal:noise ratio for the PET data; d) the 110-min half-life of fluorine-18, which permits long dynamic acquisition studies of 3 h or more; and e) a linear rate of radiotracer washout and rapid attainment of kinetic equilibrium, which provide a stable baseline for 140 min or greater for conducting pharmacological challenge studies following the IV injection of a no-carrier-added dose of [^{18}F]FCP. Because of these properties of [^{18}F]FCP, we were able to inject the indirect dopamine agonists 40 min after the start of the PET study, thereby allowing us to measure both the duration and magnitude of dopamine release.

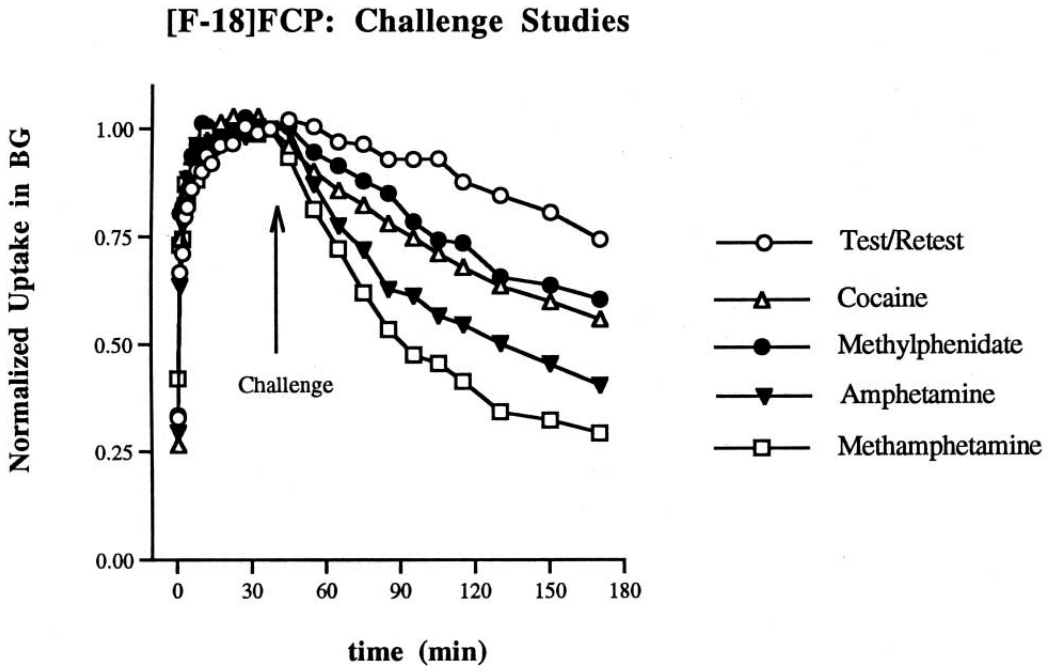


FIG. 4. Psychostimulant challenge studies in a rhesus monkey following a no-carrier-added dose of [^{18}F]FCP. The baseline tissue–time activity curve represents the averaged values from the test/retest studies in this subject. The test/retest variability in this rhesus monkey was ~10%.

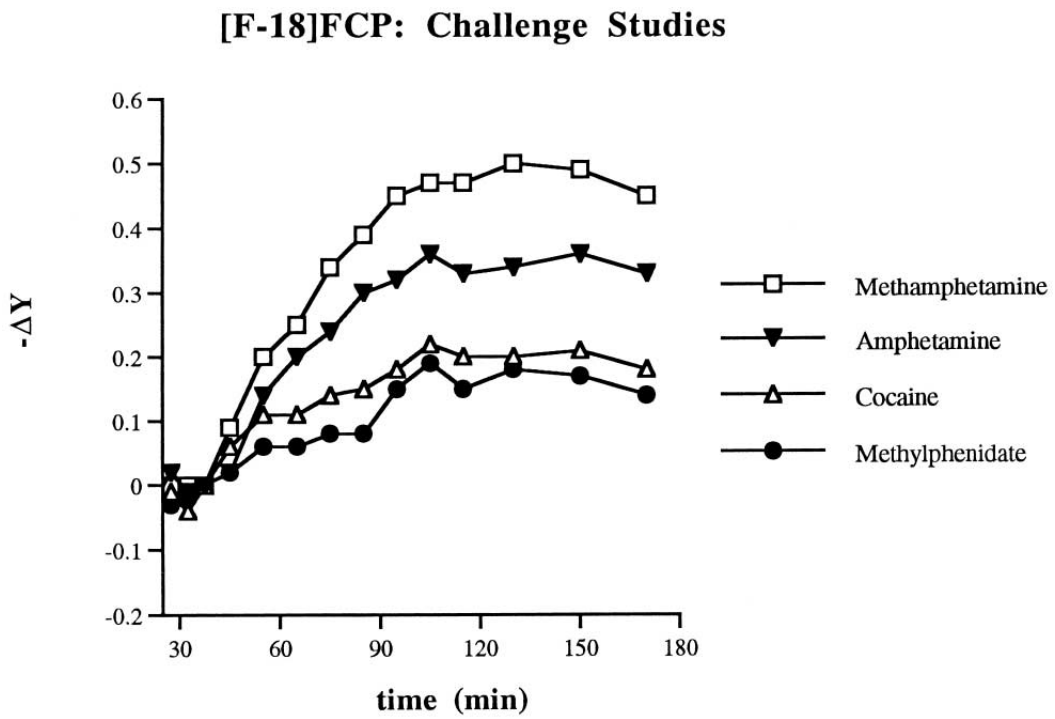


FIG. 5. Differences ($-\Delta Y$) in normalized uptake from control studies (test/retest) vs. time from the psychostimulant challenge studies. The plateau phase occurs when the rate of washout in the challenge study is equal to that in the baseline study, and represents the point in time when the ability of the psychostimulant to elevate synaptic dopamine levels, to the extent that there is competition with [^{18}F]FCP for D_2 receptors, has subsided.

The psychostimulants used in the current study were amphetamine, methamphetamine, and cocaine. These agents were chosen because extensive microdialysis data exist documenting the ability of each compound to elevate synaptic dopamine levels in both rodents (9,15) and nonhuman primates (32,39). Methylphenidate was also included in this study because this drug, which is widely prescribed by primary care physicians to treat attention deficit disorder, has been shown to display behavioral effects similar to those of amphetamine and cocaine. In fact, methylphenidate is included with methamphetamine, amphetamine, and cocaine as Schedule II drugs under the Controlled Substances Act of 1970, and an increase in IV methylphenidate abuse was recently reported in several major metropolitan areas (35).

In the present study, in vivo displacement studies of [^{18}F]FCP were conducted in a rhesus monkey that was shown to have a test/retest variability of ~10% (23). This indicates that differences in the washout kinetics of [^{18}F]FCP under conditions of psychostimulant challenge relative to the averaged tissue–time activity curve from the test/retest studies are not attributed to experimental error intrinsic to PET. The tissue–time activity curves of the PET data are represented as

TABLE 2

TISSUE CONCENTRATIONS OF [^{18}F]FCP IN THE BASAL GANGLIA AND THE CEREBELLUM

	% Injected dose/cm^3 tissue							
Study	BG (40 min)	Δ	BG (180 min)	Δ	Cb (40 min)	Δ	Cb (180 min)	Δ
Test	0.0718	—	0.0449	—	0.0168	—	0.0089	—
Retest	0.0688	—	0.0595	—	0.0190	—	0.0098	—
Average	0.0703	—	0.0522	—	0.0179	—	0.0094	—
Amph	0.0642	−8.7%	0.0260	−50.2%	0.022	+24.0%	0.0123	+32.0%
Cocaine	0.0531	−24.5%	0.0296	−43.3%	0.0169	−5.6%	0.0102	+9.1%
MP	0.0616	−12.4%	0.0373	−28.5%	0.0180	+0.6%	0.0096	+2.7%
Meth	0.0587	−16.5%	0.0172	−67.1%	0.0233	+30.0%	0.0121	+29.4%

Δ = (challenge − average)/average × 100%. BG, basal ganglia; Cb, cerebellum; Amph, *d*-amphetamine; Cocaine (−) cocaine; MP, methylphenidate; Meth, *d*-methamphetamine.

the normalized uptake of the radiotracer in the BG, which was obtained by dividing the % I.D./cm^3 at each time point by the value obtained in frame 15, which corresponds to the data point immediately prior to the administration of the challenge agent. This form of data representation was chosen to eliminate differences in the tissue–time activity curves caused by a "shift" in the peak tracer accumulation in both the BG and Cb due to a differential response to the anesthetic (see Table 2). This normalization process simply represents a scaling factor that places the tissue–time activity curves at the same reference point on the ordinate immediately prior to the challenge injection (i.e., Y = 1.00 at 40 min postinjection of [^{18}F]FCP).

The results of the challenge studies with four psychostimulants indicate that there is a dramatic increase in the rate of washout of [^{18}F]FCP from the BG relative to the test/retest baseline studies. The ability of a psychostimulant to displace [^{18}F]FCP from the BG is consistent with the ability of the drug to elevate synaptic dopamine levels. For example, both methamphetamine and amphetamine caused a more rapid rate of [^{18}F]FCP washout in comparison with cocaine; microdialysis studies in both rodents and nonhuman primates indicate that methamphetamine and amphetamine induce increases in synaptic dopamine levels (measured as the % increase over baseline) much higher than that observed with cocaine (4,9). These results are consistent with behavioral assays showing methamphetamine to be more potent than cocaine in increasing locomotor activity (4), and with *d*-amphetamine being more potent than cocaine in self-administration studies (5,17). In the present study, there appears to be little difference between cocaine and methylphenidate, because both the normalized tissue–time activity curves (Fig. 4) and the plot of $-\Delta Y$ vs. time (Fig. 5) are similar for the two drugs. This was somewhat unexpected, because PET studies with [^{11}C]cocaine and [^{11}C]methylphenidate indicated that whereas both compounds had an identical accumulation in the BG in humans, the rate of washout of methylphenidate from this region was much slower than that of cocaine (46). Microdialysis studies directly comparing the effects of methylphenidate and cocaine on dopamine release rates could provide an explanation for this discrepancy.

The results of the current study also indicate that the duration of the effect, as measured by the time necessary to reach the plateau phase in the plot of $-\Delta Y$ vs. time, was the same for each psychostimulant. In each case, the plateau phase was reached by 100 min post-IV injection of [^{18}F]FCP, which corresponds to 60 min post-IV injection of the psychostimulant, suggesting that both cocaine and amphetamine have a similar duration of action. Although this observation is contrary to microdialysis studies employing subcutaneous injections of the psychostimulant (9), our results are consistent with the dynamics of increased dopamine levels observed in microdialysis studies where the dose of the drug was given intravenously (31). Thus, it appears that the present PET methodology can provide information about the magnitude and duration of dopaminergic function under conditions that enhance dopamine release.

Although the results of the current experiments suggest that these challenge studies are measuring the dynamics of the interaction of synaptic dopamine with D_2 receptors, an alternative explanation that could be considered is the effect of the psychostimulant on cerebral blood flow. Because washout of the radiotracer from the region of interest is dependent on cerebral blood flow, it is plausible that the changes observed in the challenge PET studies may be caused by a psychostimulant-induced increase in cerebral perfusion. However, other experimental evidence suggests that the above effects are not caused by changes in cerebral blood flow. Acute exposure of humans to both methylphenidate and cocaine resulted in a significant decrease in cerebral blood flow (36,47,49), which, under the current experimental paradigm, would have resulted in a *decrease* in the rate of washout of [^{18}F]FCP from the BG, an effect opposite what we observed. Another study in rodents revealed that both methamphetamine and cocaine caused an increase in cerebral blood flow; however, this effect was transient and cerebral blood flow returned to baseline levels within a few minutes following the intravenous injection of either drug (33). Our results, which revealed an effect that persisted for 60 min post-IV injection of the drug, are more consistent with the microdialysis studies measuring synaptic dopamine content following an intravenous administration of a psychostimulant. It is also clear that the psychostimulants are not directly competing with [^{18}F]FCP for D_2 sites, because amphetamine, cocaine, and methylphenidate have a low affinity for this dopamine receptor subtype (16,28,40).

Previous studies have revealed that acute amphetamine in vitro (42) and chronic amphetamine in vivo (52) cause a time-dependent subsensitivity of mesolimbic dopaminergic neurons that is characterized by a diminished response to amphetamine. Similar results have been reported for chronic cocaine treatment in vivo (14). The results of the current study suggest that PET studies with [^{18}F]FCP using the challenge procedure described above may provide a noninvasive method for studying the longitudinal effects of psychostimulants on dopaminergic terminal activity in nonhuman primate models of substance abuse and in humans with a history of psychostimulant abuse.

In conclusion, the present results suggest that PET studies with [^{18}F]FCP may provide a means of studying the dynamics of the interaction between psychostimulant-induced dopamine levels and postsynaptic D_2 receptors. The relatively long data acquisition period (180 min), the stable baseline due to the linear rate of radiotracer washout from the BG, and the high signal:noise ratio of [^{18}F]FCP indicate that this is a suitable radiotracer for making these measurements. Furthermore, this experimental paradigm may be useful in identifying whether changes in dopamine release dynamics occur in subjects that have a history of psychostimulant abuse.

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