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Microdialysis and SPECT Measurements of Amphetamine-Induced Dopamine Release in Nonhuman Primates

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ABSTRACT The competition between endogenous transmitters and radiolabeled ligands for in vivo binding to neuroreceptors might provide a method to measure endogenous transmitter release in the living human brain with noninvasive techniques such as positron emission tomography (PET) or single photon emission computerized tomography (SPECT). In this study, we validated the measure of amphetamine-induced dopamine release with SPECT in nonhuman primates. Microdialysis experiments were conducted to establish the dose-response curve of amphetamine-induced dopamine release and to document how pretreatment with the dopamine depleter alpha-methyl-para-tyrosine (αMPT) affects this response. SPECT experiments were performed with two iodinated benzamides, [^{123}I]IBZM and [^{123}I]IBF, under sustained equilibrium condition. Both radiotracers are specific D_2 antagonists, but the affinity of [^{123}I]IBZM (K_D = 0.4 nM) is lower than that of [^{123}I]IBF (K_D = 0.1 nM). With both tracers, we observed a prolonged reduction in binding to D_2 receptors following amphetamine injection. [^{123}I]IBZM binding to D_2 receptors was more affected than [^{123}I]IBF by high doses of amphetamine, indicating that a lower affinity increases the vulnerability of a tracer to endogenous competition. With [^{123}I]IBZM, we observed an excellent correlation between reduction of D_2 receptor binding measured with SPECT and peak dopamine release measured with microdialysis after various doses of amphetamine. Pretreatment with αMPT significantly reduced the effect of amphetamine on [^{123}I]IBZM binding to D_2 receptors, confirming that this effect was mediated by intrasynaptic dopamine release. Together, these results validate the use of this SPECT paradigm as a noninvasive measurement of intrasynaptic dopamine release in the living brain. **Synapse 25:1–14, 1997.**

INTRODUCTION

The competition between endogenous transmitters and radiolabeled ligands for in vivo binding to neuroreceptors might provide a method to measure endogenous transmitter release in the living human brain with noninvasive techniques such as positron emission tomography (PET) or single photon emission computerized tomography (SPECT). Such a method would be very valuable to study synaptic activity in specific anatomical and chemical systems in various neuropsychiatric conditions.

Competition between dopamine (DA) and radiolabeled ligands for binding to DA D_2 receptors is well documented in rodents. Amphetamine, which releases DA and thereby increases endogenous DA concentration (Kuczenski and Segal, 1989; Sharp et al., 1987), reduced the in vivo binding of the D_2 agonist, [^{3}H]N-propylnorapomorphine (Köhler et al., 1981; Ross and

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Jackson, 1989b), and of the D_2 antagonist [^{3}H]raclopride (Ross and Jackson, 1989a; Young et al., 1991). Reduced in vivo accumulations of D_2 tracers were also reported following pretreatment with the DA uptake inhibitors amfonelic acid and methylphenidate and with the DA precursor L-DOPA (DeJesus et al., 1986; Ross and Jackson, 1989b). The opposite effect (i.e., increased tracer accumulation) was induced by drugs that decrease DA endogenous concentration, such as reserpine and γ-butyrolactone (Ross, 1991; Ross and Jackson, 1989b; Seeman et al., 1989; Van der Werf et al., 1986; Young et al., 1991).

Similar observation were reported using PET and SPECT. Logan et al. (1991) reported that the rate of uptake of the PET D_2 tracer [^{18}F]N-methylspiroperidol was decreased in baboons pretreated with amphetamine (1 mg/kg). Innis et al. (1992) and Kessler et al. (1993b) showed that the striatal washout rates of the D_2 tracers [^{123}I]IBZM and 5-[^{18}F]fluoropropylepidepride were increased by amphetamine (0.3–1.5 mg/kg) in baboons. Dewey et al. (1993) reported decreased binding of [^{11}C]raclopride in baboons pretreated with amphetamine (1 mg/kg). In humans, Farde et al. (1992) showed that the in vivo binding of [^{11}C]raclopride was decreased by 6–16% after oral administration of amphetamine (30 mg). Volkow et al. (1994) reported a 23 ± 15% decrease in [^{11}C]raclopride specific binding in humans after i.v. methylphenidate (0.5 mg/kg). Laruelle et al. (1995) documented a 15 ± 4% decrease in [^{123}I]IBZM specific binding in humans after i.v. amphetamine (0.3 mg/kg).

Together, these data suggest the possibility of noninvasive measurement of changes in endogenous DA concentration in the living human brain following pharmacological challenge tests. However, the relationship between changes in endogenous DA concentration and changes in tracer binding parameters remain to be clarified. For example, Dewey et al. (1993) reported no difference in the decrease of [^{11}C]raclopride binding induced by 1.5 and 3.0 mg/kg GBR-12909 in baboons. Similarly, Volkow et al. (1994) failed to observe significant differences in the decrease of [^{11}C]raclopride binding following 0.25 vs 0.5 mg/kg methylphenidate inhumans. These results raised concerns about the sensitivity of this paradigm to changes in DA synaptic concentration.

To assess the validity of this noninvasive imaging technique as a measure of changes in endogenous DA concentration, the following investigations were conducted: 1) we studied the relationship between changes in DA concentration (measured with microdialysis) and changes in binding parameters (measured with SPECT) after various doses of amphetamine; 2) we investigated the role of tracer affinity in vulnerability to endogenous competition by comparing the amphetamine effect on the in vivo binding of two SPECT D_2/D_3 radiotracers, [^{123}I]IBF (K_D = 0.1 nM) and [^{123}I]IBZM (K_D = 0.4 nM) (Kung et al., 1988b; Kung et al., 1990); 3) we assessed the reproducibility of the amphetamine effect on tracer binding; 4) to corroborate that competition with endogenous DA is the mechanism underlying the amphetamine effect on radiotracer binding, we assessed the effect of the DA synthesis inhibitor alpha-methyl-paratyrosine (αMPT) on both microdialysis and SPECT measurements; 5) to understand observed discrepancies between the time course of SPECT and microdialysis measurements, we further investigated the kinetics of [^{123}I]IBZM displacement by performing experiments with subsaturating doses of the D_2 antagonist raclopride.

All SPECT experiments were conducted using the radiotracer constant infusion technique previously developed (Laruelle et al., 1994a,b). This constant infusion technique allows for control of potential effects of amphetamine on blood flow or tracer peripheral clearance which could bias the SPECT measurement of amphetamine-induced changes in binding parameters.

METHODS

General design

Microdialysis experiments (n = 12) were performed in two male vervets, 5–7 kg, referred to as vervets A and B. SPECT scanning experiments (n = 32) were performed in six ovariectomized female baboons, 10–12 kg, referred to as baboons A–E. Experiments were carried out in different species because the smaller size of the vervet's head was well adapted to the stereotaxic frame used for probe placement, and the larger size of the baboon's brain minimized partial voluming effects in the SPECT images.

Repeated microdialysis experiments on the same animal were separated by at least 3 weeks. Microdialysis experiments were first performed on vervet A to measure extracellular DA release after various doses of amphetamine (0.03, 0.27, 0.68, 1.0, and 1.5 mg/kg i.v. bolus). Vervet B was then used to measure the effect of αMPT pretreatment on basal DA extracellular concentration and amphetamine-induced DA release. This animal underwent seven experiments with 0.5 mg/kg amphetamine (three experiments in control condition and four experiments with αMPT pretreatment).

SPECT experiments took place over a 2 year period. Baboons A, B, and C were used for the [^{123}I]IBF dose-response experiments. Baboons A, B, and D were used for the [^{123}I]IBZM dose-response experiments. Thus, baboons A and B participated in both groups of dose-response experiments. Experiments with αMPT pretreatment were performed with [^{123}I]IBZM in baboons A and D. Experiments with low dose raclopride displacement were performed with [^{123}I]IBZM in baboon E.

For the [^{123}I]IBF dose response study (n = 17), each animal first underwent a control experiment (absence of amphetamine injection). During the control experi-

ments, baboons were injected with a receptor saturating dose of raclopride (1 mg/kg) to validate the use of the occipital activity as an estimate of the striatal nondisplaceable activity (= free plus nonspecifically bound activity). Following the control experiment, each animal underwent four experiments with amphetamine, at doses of 0.1, 0.3, 0.5, and 1 mg/kg. Repeated amphetamine administration in rodents induces an increase in amphetamine-induced DA release, a phenomenon referred to as sensitization (Paulson and Robinson, 1995; Robinson and Becker, 1986). To minimize sensitization-induced bias of the dose-response curve, the sequences of the amphetamine doses were random, and the experiments took place over a 12 month period, with a minimum of 3 weeks between each experiment.

The [^{123}I]IBZM dose-response study (n = 12) was initiated 6 months after completion of the [^{123}I]IBF study. Baboon C was not available for the [^{123}I]IBZM study. Thus, the [^{123}I]IBZM experiments were performed on baboons A, B, and D. Baboons A and B first underwent a control experiment with raclopride injection. Each animal then underwent three experiments with amphetamine doses of 0.3, 0.5, and 1 mg/kg. These experiments took place, in a random order, over a period of 6 months.

Reproducibility of the SPECT measurement of the amphetamine effect was assessed by repeating the 0.3 and 0.5 mg/kg amphetamine doses on baboon B with [^{123}I]IBF and the 1 mg/kg dose on baboon D with [^{123}I]IBZM. αMPT pretreatment experiments (n = 2) were performed on baboon A and D using [^{123}I]IBZM as the tracer and amphetamine doses of 0.3 and 0.5mg/kg, respectively. Finally, the kinetics of [^{123}I]IBZMdisplacement after low doses of raclopride injection(4 μg/kg and 8 μg/kg) was studied in baboon E.

Microdialysis experiments

Microdialysis experiments were performed as previously described (Iyer et al., 1995). Surgical placement of the cannulae and microdialysis experiments were performed under isoflurane anesthesia (1.5–2.5%) with temperature maintained at 37°C as previously described (Laruelle et al., 1994c). Stereotaxic placement of chronic guide cannulae was performed using a magnetic resonance imaging (MRI) directed procedure (Iyer et al., 1995; Saunders et al., 1990; Wang et al., 1990). In brief, an animal was placed in a nonferromagnetic stereotaxic frame, which can be aligned with the reference planes of the MR Imager (Sigma III; General Electric, Milwaukee, WI). Vitamin E–filled ear bars provided anterior-posterior reference coordinates, while the superior sagittal sinus provided a medial-lateral reference. Bilateral craniotomies were made overlying the caudate nucleus and exposing the superior sagittal sinus, and guide cannulae containing six placement sites each were cemented into place using dental acrylic adhesive. Four to six stainless steel skull screws served as anchor points. The dura was left intact during this procedure to reduce the risk of central infection.

Microdialysis probes of a concentric design, similar to others previously reported (Johnson and Justice, 1983), were constructed as previously described (Bradberry and Roth, 1989) using Cuprophan (Enka, Germany) hollow fibers (300 μm i.d., 330 μm o.d.) housed in a section of 23 gauge (0.64 mm o.d.) hypodermic tubing. The fiber extended 3.2–5.6 mm beyond the tip of the tubing, exposing an active length determined from the MR image, as was the depth of placement. The probe was glued into a disk which could be locked into place over the guide cannula. Perfusion buffer (in mM: KCl 2.4, NaCl 137, $CaCl_2$ 1.2, $MgCl_2$ 1.2, NaH_2PO_4 0.9, ascorbic acid 0.075; pH 7.4) was pumped through a section of vitreous silica (73 μm i.d., 140 μm o.d.; Polymicro Technologies, Phoenix, AZ) which extended to the tip of the hollow fiber. Ethylene oxide–sterilized probes were lowered through the chronic guide cannulae under sterile conditions. Collection periods were fixed at 20 min intervals with the flow rate set at 2 μl/min. The probes were calibrated in vitro at the termination of the experiment, and the resulting percentage recoveries were used to correct for differences between probes. A fresh site was used for each probe implantation. For αMPT microdialysis experiments (n = 4), a total dose of 235 mg/kg of αMPT was given as follows: a bolus of 75 mg/kg was given over 10 min, followed by an infusion of 40 mg/kg/h for 4 h. Amphetamine (0.5 mg/kg) was injected at the end of the αMPT infusion.

For liquid chromatographic determination of neurotransmitter levels in the brain dialysates, microbore columns (100 × 1 mm i.d. packed with 3 μm C-18 particles; Bioanalytical Systems, West Lafayette, Washington) were used in conjunction with a thin-layer amperometric detection electrode assembly and potentiostat (E_{app}: +0.6 V vs. Ag/AgCl reference; Bioanalytical Systems, West Lafayette, Washington). A pneumatic fluid–displacement pump (Bradberry et al., 1991) was used to pump the mobile phase through the column. The composition of the mobile phase was 0.07 M NaH_2PO_4, 440 mg/l sodium octanesulphonate, 0.1 mM disodium EDTA, and 60 ml/l acetonitrile. The pH was adjusted to 5.0. The limit of detection attainable was routinely 1–2 fmol injected. Statistical comparisons were done with data expressed in fentomole per microliter of dialysate, corrected for in vitro recovery.

SPECT experiments

[^{123}I](*S*)-5-iodo-7-*N*-[1-ethyl-2-pyrrolidinyl)methyl-]carboxamino-2, 3-dihydrobenzofuran ([^{123}I]IBF) and [^{123}I](*S*)-(−)-3-iodo-2-hydroxy-6-methoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl]benzamide ([^{123}I]IBZM) are both iodinated benzamides with high affinities for D_2 and D_3 receptors and negligible affinity for D_4 receptors (in this

paper, we use the term D_2 to designate D_2 and D_3 receptors). The affinity of [^{123}I]IBF for D_2 receptors (K_D at 37°C = 0.11 nM) is higher than [^{123}I]IBZM (0.43 nM) (Kung et al., 1988b; Kung et al., 1990). The lipophilicity of [^{123}I]IBF (log k_w at pH 7.5 = 2.32) is lower than [^{123}I]IBZM (2.75) (Kessler et al., 1991). Labeling of [^{123}I]IBF and [^{123}I]IBZM was performed as previously described (Al-Tikriti et al., 1994; Kung et al., 1988a; Kung and Kung, 1990, Murphy et al., 1990). Specific activity was higher than 5,000 Ci/mmole, so experiments were conducted at tracer doses and negligible tracer receptor occupancy.

Baboons were anesthetized using the same protocol as for microdialysis experiments. Two intravenous indwelling catheters were used, one for radiotracer infusion and the other for amphetamine injection and hydration (0.9% NaCl at 50 ml/h). Radiotracer administration was started at least 90 min after the initiation of the anesthesia. The radiotracer was given as an initial bolus immediately followed by constant infusion for the duration of the study (8 h). The radiotracer administration protocol was designed by computer simulation based on kinetic parameters previously reported (Laruelle et al., 1994b). The aim of this tracer administration protocol was to rapidly establish a state of tracer equilibrium in the plasma and in the brain and to sustain this state for the duration of the study (Laruelle et al., 1994a,b). For [^{123}I]IBF, a bolus of 3.5 ± 0.9 mCi was immediately followed by a constant infusion at a rate of 1.7 ± 0.5 mCi/h for 8 h. The average bolus to hourly infusion ratio was 1.9 ± 0.3 h, and the total administered dose was 17 ± 3.6 mCi. For [^{123}I]IBZM, a bolus of 2.8 ± 1.5 mCi was immediately followed by a constant infusion at a rate of 0.83 ± 0.39 mCi/h for 8 h. The average bolus to hourly infusion ratio was 3.4 ± 0.2 h, and the total administered dose was 9.5 ± 4.6 mCi. Thus, while significant variation in the total injected dose was allowed (depending on the labeling yield), the bolus to infusion ratio was kept constant for each tracer. This ratio was higher for [^{123}I]IBZM than for [^{123}I]IBF because of the slower peripheral clearance of [^{123}I]IBZM compared to [^{123}I]IBF. Unless otherwise specified, times correspond to times elapsed since the beginning of the tracer administration.

Amphetamine was injected i.v. at 240 min as a 30 sec bolus in 5 ml 0.9% NaCl. For αMPT experiments (n = 2), a total dose of 240 mg/kg of αMPT was given as follows: a bolus of 80 mg/kg was given over 10 min at 60 min prior to the beginning of the [^{123}I]IBZM infusion, followed by an infusion of 40 mg/kg/h for 4 h that was started at the same time as the [^{123}I]IBZM infusion. Amphetamine was thus given at the end of the αMPT infusion. For low dose raclopride experiments (n = 2), raclopride was given at 180 min as an i.v. bolus at doses of 4 and 8 μg/kg, respectively.

Data were acquired with the CERASPECT camera (Digital Scintigraphics, Waltham, MA). Scans of 5 min ([^{123}I]IBF) or 10 min ([^{123}I]IBZM) duration were acquired in the continuous mode for 480 min. Given a 37 sec delay between acquisitions, 90 and 45 scans were acquired per [^{123}I]IBF and [^{123}I]IBZM experiments, respectively. The shorter acquisition time of [^{123}I]IBF compared to [^{123}I]IBZM was possible because of the superior target to background ratio achieved with [^{123}I]IBF compared to [^{123}I]IBZM. Images were reconstructed, attenuation-corrected, and reoriented as previously described (Laruelle et al., 1994c). The three images with highest striatal activities were identified and summed. Three standard regions of interest were positioned on this summed image: right and left striatum (376 mm^2) and occipital pole (919 mm^2). Right and left striatum were averaged.

Equilibrium analysis was performed to evaluate the effect of amphetamine on the D_2 binding potential (BP). According to the original definition of Mintun et al., (1984), BP (ml/g) is equal to the ratio of receptor density (B_{max}, nM or pmol/g of brain tissue) to the equilibrium dissociation constant (K_D, nM or pmol/ml of plasma). According the Michaelis-Menten equation, under tracer conditions (negligible receptor occupancy), the BP is also equal to the ratio of the specifically bound (B) to the free ligand concentration (F).

$$BP = B_{max}/KD = B/F \quad (1)$$

Passive diffusion being the mechanism of transport of the tracer across the blood-brain barrier, the concentration of free tracer in the plasma and the brain are equal at equilibrium. It follows that, when equilibrium is reached during a constant infusion of a high specific activity radiotracer, BP is equal to the ratio of the specific binding to the plasma steady-state concentration of the free (unbound to plasma protein) parent compound (Kawai et al., 1991; Laruelle et al., 1994a). Similarly, the equilibrium volume of distribution of the nondisplaceable compartment (V_2) is given by the equilibrium ratio of the nondisplaceable activity (including free and nonspecifically bound activity) to the plasma free parent compound concentration.

The equilibrium specific to nonspecific partition coefficient is denoted V_3'' and corresponds to the ratio of BP to V_2 (Laruelle et al., 1994d). Assuming that the total occipital activity is equal to the striatal nondisplaceable activity, V_3'' can be calculated at equilibrium as

$$V_3'' = BP/V_2 = [(S-O)/F]*F/O = (S-O)/O \quad (2)$$

where S is the equilibrium total striatal activity and O is the equilibrium occipital activity. The amphetamine-induced decrease in D_2 receptors BP was calculated from the relationship

$$BP_a/BP_b = V_3''_a/V_3''_b = ((S_a-O_a)/O_a)/((S_b-O_b)/O_b) \quad (3)$$

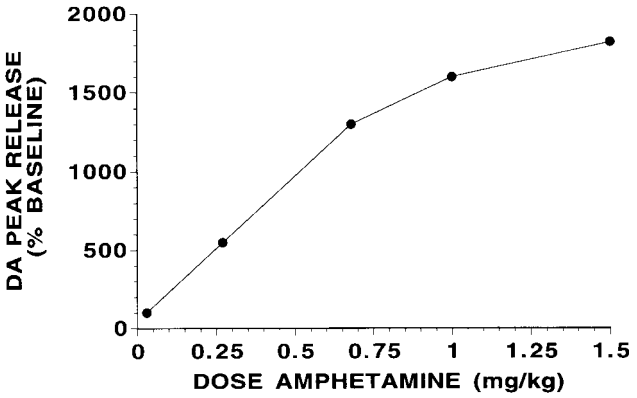

Fig. 1. Dose-response curve for maximal amphetamine-induced elevations in extracellular DA measured with microdialysis in one vervet. Values are expressed as percent of preamphetamine baseline values.

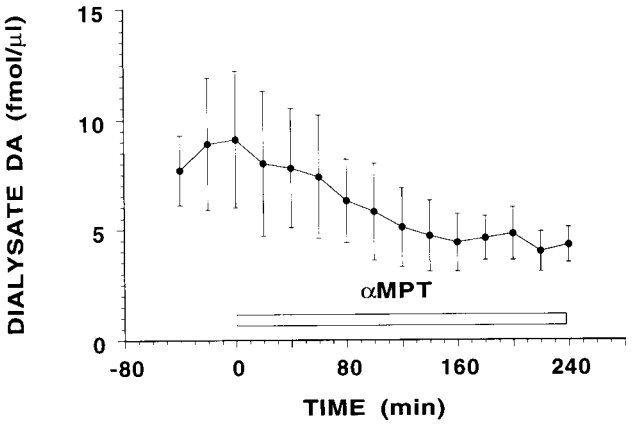

Fig. 2. Time course of effect of αMPT upon basal DA. The cumulative dose of i.v. αMPT was 235 mg/kg, consisting of an initial dose of 75 mg/kg followed by an infusion of αMPT at a dose of 40 mg/kg/h given over 4 h. Values are the mean ± SEM of four experiments. The αMPT infusion reduced basal DA level to 47% of pre-αMPT values.

where the subscript *a* denotes measures after amphetamine and *b* denotes measures before amphetamine. The equivalence between the reduction in D_2 BP and D_2 V_3'' in equation 3 was derived from equation 2, assuming that V_2 is unaffected by amphetamine injection. Amphetamine-induced decrease in D_2 BP was expressed in percentage of baseline BP. Potential changes in plasma clearance induced by amphetamine would result in changes in the plasma steady-state level that would equally affect both specific and nondisplaceable equilibrium activity level. Thus, the use of V_3'' as the outcome measure corrects for potential changes in peripheral clearance following amphetamine.

Statistical analysis

Values were expressed as mean ± SD, unless otherwise specified. Effects of αMPT infusions on preamphetamine DA dialysate concentration and on amphetamine-induced DA release were assessed by repeated measures ANOVA and two-way repeated measuresANOVA, respectively. Between animal differences in baseline V_3'' were assessed by ANOVA. The effect of animal and amphetamine dose on tracer displacement was investigated by two-way ANOVA with animal and dose as factors. Reproducibility of tracer displacement was evaluated with the intraclass correlation coefficient (Kirk, 1982).

Drugs

Amphetamine and αMPT were purchased from Sigma (St. Louis, MO). Doses refer to the sulfate salt of D-amphetamine and the methyl ester of αMPT.

RESULTS

Microdialysis experiments

Dose response curve

Injection of amphetamine induced a rapid increase of extracellular DA that peaked in the first sample collected after the amphetamine injection (0–20 min). At peak value, DA was increased by 100, 549, 1,300, 1,600, and 1,824% above baseline values after 0.03, 0.27, 0.68, 1.0 and 1.5 mg/kg amphetamine, respectively (Fig. 1). Peak DA concentration was followed by a rapid decline. In the 60–80 min postamphetamine collection, DA concentration represented 50 ± 6% of peak value. In the 100–120 min postamphetamine collection (end point of data collection), extracellular DA concentration was still elevated as compared to baseline but at a level much lower than peak level (34 ± 5.3% of peak value).

αMPT pretreatment

From the dose-response curve, a dose of 0.5 mg/kg amphetamine was chosen to determine the effect of αMPT pretreatment. This dose was administered four times to vervet B to establish the response to control conditions. Basal dialysate DA in these control experiments was 17.7 ± 9.5 fmol/µl (values corrected for probe recovery and expressed as mean ± S.E.M.; n = 4) and was significantly enhanced by 0.5 mg/kg amphetamine to a maximal value of 522 ± 205 fmol/µl (0–20 min postamphetamine collection).

In three separate experiments, the animal was pretreated with αMPT prior to the amphetamine injection. The mean pre-αMPT baseline DA concentration was lower in these experiments (8.3 ± 2.4 fmol/µl; n = 3) than in the control experiments (17.7 ± 5.3 fmol/µl; n = 4), but this difference was not statistically significant ($P = 0.22$, *t*-test). During the αMPT infusion (4 h), a significant decrease in basal DA was observed, from 8.3 ± 2.4 fmol/µl before αMPT to 4.3 ± 0.8 fmol/µl at the end of the infusion (Fig. 2) ($P = 0.002$, repeated measures ANOVA).

After αMPT, amphetamine (0.5 mg/kg) increased extracellular DA from 4.3 ± 0.8 fmol/µl to 89 ± 28 fmol/µl. The amphetamine effect observed after αMPT

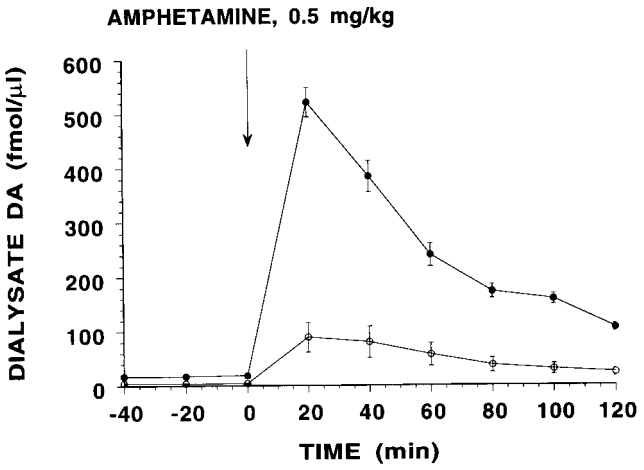

Fig. 3. Impact of αMPT pretreatment upon the ability of 0.5mg/kg amphetamine to increase dialysate DA, presented as dialysate concentration of DA (mean ± SEM, corrected for in vitro probe recovery). All experiments were performed in the same animal. After αMPT, amphetamine-induced DA release (open circles, n = 4) was significantly lower than in control conditions (closed circles, n = 3, $P = 0.009$, two-way repeated measures ANOVA).

pretreatment was significantly attenuated compared to the amphetamine effect observed in control conditions (Fig. 3) ($P = 0.009$, two-way repeated measures ANOVA). Peak postamphetamine dialysate measurements were 522 ± 205 fmol/µl in control conditions vs. 89 ± 28 fmol/µl in αMPT pretreatment conditions. Therefore, αMPT reduced the absolute postamphetamine peak DA concentration by 83%. Expressed in relative terms (% increase above baseline), amphetamine increased DA by 2,448 ± 740% in control conditions vs. 1,993 ± 511% after αMPT (i.e., αMPT reduced the amphetamine effect by 19%).

SPECT experiments

[^{123}I]IBF dose response curve

Control experiments. The bolus plus constant infusion protocol induced stable levels of both striatum and occipital activities (Fig. 4A). Activity levels stabilized at 120–180 min and remained constant thereafter. V_3'' measured from 360–420 min was similar to V_3'' measured from 180–240 min (changes of −6, 0, and −3%) (Table I). Raclopride injection at a receptor saturating dose (1 mg/kg) induced displacement of striatal activity to levels observed in the occipital region. These experiments established that a stable baseline was obtained with this tracer administration schedule and that occipital activity could be used as an estimate of striatal nondisplaceable activity.

Amphetamine experiments. Baseline V_3'' values were in the range of values previously reported (Laruelle et al., 1994b). We observed significant differences in baseline V_3'' between the animals, baboon A showing smaller baseline V_3'' (1.73 ± 0.08, n = 5) than baboon B (1.99 ± 0.15, n = 5) and baboon C (1.91 ± 0.13, n = 5, $P = 0.025$). The between experiment reproducibility of baseline V_3'' measurement had a coefficient of variation of 7 ± 1%.

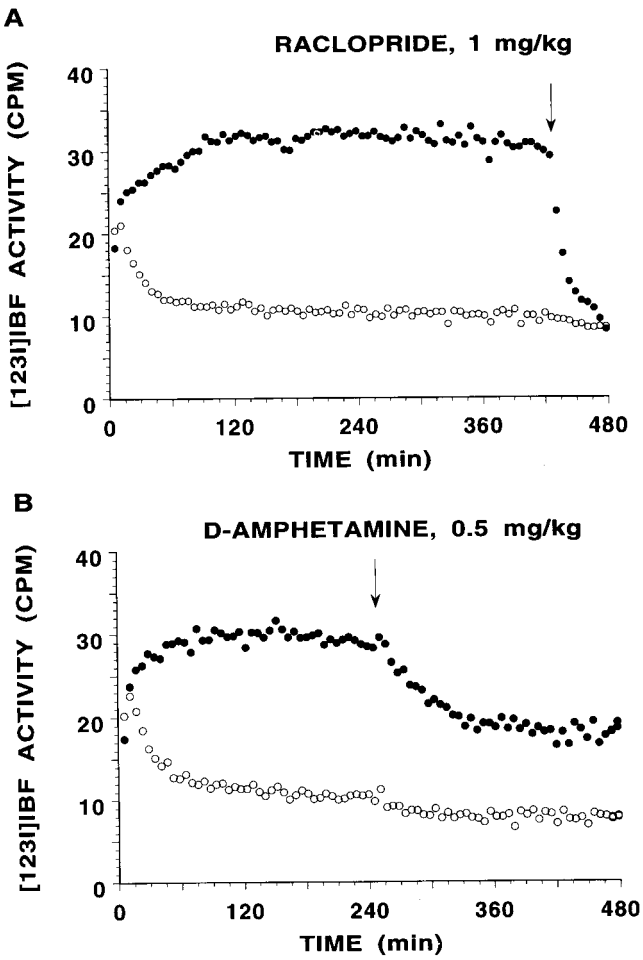

Fig. 4. Striatal (closed circles) and occipital (open circles) activities during [^{123}I]IBF bolus plus constant infusion in baboon C. **A:** Control experiment. Baboon C was injected with 4 mCi, followed by constant infusion at rate of 1.8 mCi/h for 480 min. Data were acquired continuously on the CERASPECT camera (5 min acquisition time). Regional activities stabilized around 180 min. The specific to nonspecific partition coefficient (V_3''), calculated as (striatal − occipital)/occipital activities ratio measured from 180–240 min was 2.10 and remained stable thereafter. Injection of a receptor saturating dose of raclopride (1mg/kg, i.v.) at 410 min (arrow) induced a decrease of striatal activity to the level observed in the occipital region, justifying the use of this region as an estimate of the striatal nondisplaceable activity. **B:** Amphetamine experiment. The experiment was repeated in the same animal under identical conditions, except that amphetamine was injected (0.5 mg/kg, i.v.) at 240 min (arrow). This injection induced a prolonged reduction in striatal activity. Postamphetamine V_3'', measured from 360–420 min (1.34), was reduced by 26% compared to baseline values, measured from 180–240 min (1.81).

The amphetamine injection (240 min) induced a prolonged decrease in striatal activity that reached a new stable level around 360 min (Fig. 4B). Striatal activity remained stable from about 360 min to the end of the experiments (480 min). Occipital activity was unaffected by low doses amphetamine (0.1 and 0.3 mg/kg) but showed a small decrease after injection of

TABLE I. Decrease V3″ during [123I]IBF constant infusion after amphetamine challenge test[1]

	Baboon A		Baboon B		Baboon C	
Challenge	Baseline V_3''	Decrease V_3'' (%)	Baseline V_3''	Decrease V_3'' (%)	Baseline V_3''	Decrease V_3'' (%)
Control	1.79	−6	2.22	0	2.10	−3
Amphetamine 0.1 mg/kg	1.62	10	1.82	8	1.96	8
Amphetamine 0.3 mg/kg	1.72	22	2.03	11	1.77	36
Amphetamine 0.5 mg/kg	1.84	25	1.88	16	1.81	26
Amphetamine 1.0 mg/kg	1.70	18	1.95	14	1.91	31

[1] V_3'' is the specific to nonspecific partition coefficient at equlibrium and is calculated as (striatum-occipital)/occipital activities at equilibrium. For each experiment, baseline V_3'' was measured from 180–240 min (i.e., before the amphetamine injection). Amphetamine was then injected at 240 min. Postamphetamine V_3'' was measured from 360–420 min. Decrease in V_3'' is expressed as % of baseline V_3''. Effects of doses and animal were investigated by two-way ANOVA. We observed significant differences between animals in the amphetamine effect (C > A > B, P = 0.023) but no differences between the three higher amphetamine doses (P = 0.89).

0.5 and 1 mg/kg amphetamine (see occipital curve on Fig. 4B). The amphetamine effect on D_2 BP was quantified by comparing postamphetamine V_3'' (measured during the last hour of the experiment [i.e., 360–420 min]) to the baseline V_3'' (measured during the hour preceding the amphetamine injection [i.e., 180–240 min]) (Table I). In the three baboons, the 0.1 mg/kg dose induced a modest decrease in D_2 BP (8.5 ± 1.5% reduction). The three higher doses induced similar and larger decrease of V_3'' (22 ± 12%, 22 ± 5.6%, 21 ± 9.3% after 0.3, 0.5 and 1.0 mg/kg doses, respectively, n = 3 per doses) (Table 1; Fig. 6). Effect of doses and animal were investigated by two-way ANOVA. We observed significant differences between animals in the amphetamine effect (C > A > B, $P = 0.023$) (Table I) but no differences between the three higher amphetamine doses ($P = 0.89$) (Table I).

[123I]IBZM dose-response curve

Control experiments. Control experiments were performed on baboon A and B. These experiments demonstrated the stability of the striatal and occipital activity during [123I]IBZM constant infusion (Fig. 5A). V_3'' values at the 360–420 min interval were similar to V_3'' values at the 180–240 min interval (change of −2 and −3%). Raclopride injection reduced the striatal activity to level close but not equal to the occipital activity (striatal to occipital ratio from 45–60 min after raclopride injection was 1.14 and 1.24 for baboon A and C, respectively). These experiments suggested that approximately 20% of the "specific" striatal activity, as estimated by the striatal minus occipital method, might represent nondisplaceable activity. In spite of this, the simple subtraction method was used in the estimation of the [123I]IBZM specific binding. Precise measurement of striatal nondisplaceable activity would have required performing raclopride displacement at the end of each experiment, which was not done, out of concern that repeated injection of this neuroleptic drug might affect DA function in these animals. This underestimation of striatal nondisplaceable binding probably induced a 10% underestimation of the amphetamine effect on D_2 V_3'' in the [123I]IBZM experiments. This phenomenon was not observed in humans, in which injection of receptor saturating doses of haloperidol (20 μg/kg) reduced the striatal activity to level observed in the occipital cortex (Seibyl et al., 1992).

Amphetamine experiments. Baseline V_3'' was markedly lower for [123I]IBZM (0.73 + 0.08, n = 11) than for [123I]IBF (1.88 ± 0.15, n = 15), as can be appreciated by comparing Figures 4 and 5. Despite this lower target to background ratio, [123I]IBZM was as effective as [123I]IBF in detecting the smaller D_2 receptor binding in baboon A (0.63 ± 0.04, n = 4) compared to baboon B (0.77 ± 0.03, n = 4, $P = 0.002$, *t*-test). In addition, the between-experiment reproducibility of baseline V_3'' measurement was comparable for [123I]IBZM (coefficient of variation of 6 ± 2%) and [123I]IBF (7 ± 1%). This reproducibility was in the usual range of reproducibility for neuroreceptor measurement with PET or SPECT (Abi-Dargham et al., 1995; Volkow et al., 1993).

Amphetamine experiments with [123I]IBZM were performed with the 0.3, 0.5, and 1.0 mg/kg doses. As observed with [123I]IBF, the amphetamine injection resulted in a reduction of [123I]IBZM striatal activity until a new stable level was established (Fig. 5B).

In contrast with [123I]IBF experiments, we observed a significant dose effect with [123I]IBZM in this dose range: the D_2 V_3'' reduction was 20 ± 5%, 28 ± 8% and 38 ± 11% after 0.3, 0.5, and 1.0 mg/kg amphetamine doses, respectively (two-way ANOVA with dose and animal as factors; dose $P = 0.016$; animal: $P = 0.029$) (Table II; Fig. 6). Thus, in contrast with [123I]IBF, [123I]IBZM displacement was larger when the amphetamine dose was increased from 0.3–1.0 mg/kg. Interestingly, the significant between-animal difference in the amphetamine effect observed with [123I]IBZM was in the same direction as the one observed with [123I]IBF: baboon A showed larger displacement than baboon B with both [123I]IBF and [123I]IBZM.

Results of the SPECT [123I]IBZM dose-response experiments were compared to microdialysis measurement of amphetamine-induced DA release (dose range 0.03–1.5 mg/kg). Since doses of amphetamine used in the microdialysis experiments were not exactly the same as the doses used in the SPECT experiments,

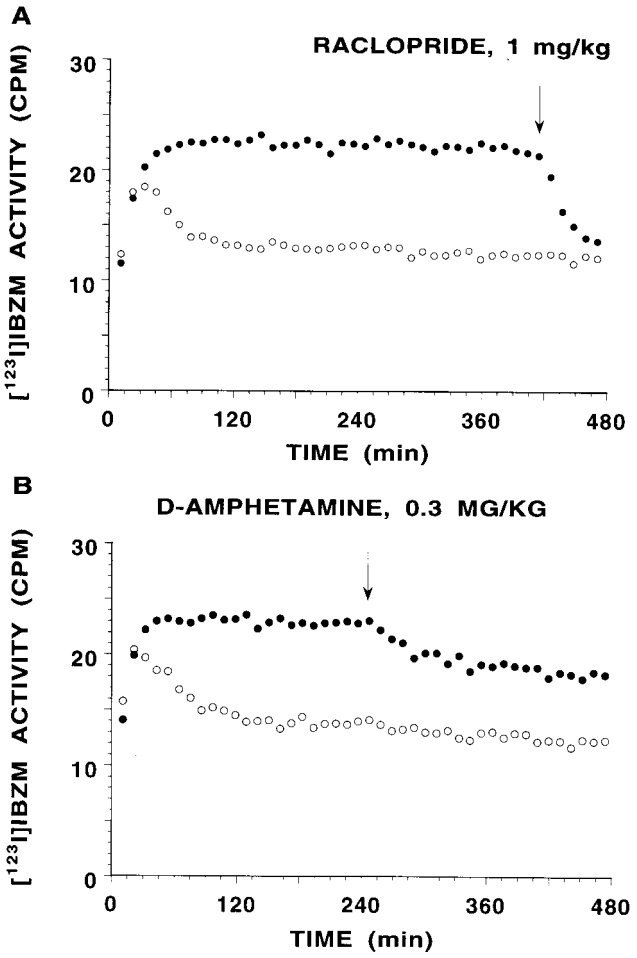

Fig. 5. Striatal (closed circles) and occipital (open circles) activities during [^{123}I]IBZM bolus plus constant infusion in baboon A. **A:** Control experiment. Baboon A was injected with 2 mCi, followed by constant infusion at rate of 0.6 mCi/h for 480 min. Data were acquired continuously on the CERASPECT camera (10 min acquisition time). Regional activities stabilized around 120 min. Comparison of Figs. 4 and 5 illustrates the lower specific to nonspecific partition coefficient (V_3'') of [^{123}I]IBZM compared to [^{123}I]IBF. Measured from 180–240 min, V_3'' was 0.68 and remained stable thereafter. Injection of a receptor-saturating dose of raclopride (1 mg/kg, i.v.) at 400 min (arrow) induced a decrease of striatal activity to the level close but not equal to the level observed in the occipital region, indicating that [^{123}I]IBZM striatal nondisplaceable activity is slightly higher than occipital activity. **B:** Amphetamine experiment. The experiment was repeated in the same animal under identical conditions, except that amphetamine was injected (0.3 mg/kg, i.v.) at 240 min (arrow). This injection induced a prolonged reduction in striatal activity. Postamphetamine V_3'', measured from 360–420 min (0.50), was reduced by 25% compared to baseline value, measured from 180–240 min (0.68).

microdialysis values at 0.3, 0.5, and 1 mg/kg were estimated by fitting measured microdialysis values to a hyperbolic function ($y = ax/[b + x]$). Expressed as increase over baseline, peak DA release was estimated as 731, 1,031, and 1,523% after 0.3, 0.5, and 1.0 mg/kg doses, respectively. A linear relationship was observed between amphetamine-induced peak DA release and amphetamine-induced [^{123}I]IBZM displacement ($r^2 = 0.99$) (Fig. 7).

Reproducibility of the amphetamine effect

Three experiments were repeated to assess the within-animal reproducibility of the amphetamine effect: the 0.3 and 0.5 mg/kg doses were repeated in baboon B using [^{123}I]IBF, and the 1 mg/kg dose was repeated with baboon C using [^{123}I]IBZM (Table III). Reproducibility was assessed by computing the test-retest difference and the intraclass coefficient of correlation. Despite the large time interval between the two test and retest (85 + 65 days), the average difference between the test and retest was only 2 ± 2% (n = 3), which corresponded to an intraclass correlation coefficient of 0.97.

αMPT pretreatment experiments

Since dose-response experiments established that [^{123}I]IBZM binding was more sensitive than [^{123}I]IBF binding to changes in endogenous DA, experiments in baboons pretreated with αMPT were performed only with [^{123}I]IBZM. Baboon A and D each underwent one SPECT experiment after pretreatment with αMPT and received amphetamine doses of 0.3 and 0.5 mg/kg, respectively. Results of these experiments (Table IV) were compared to the result of the experiment performed in control condition (non-αMPT pretreated) (Table 2).

In both animals, baseline V_3'' was increased compared to control experiments (0.85 vs. 0.66 for baboon A and 1.07 vs. 0.82 for baboon B), indicating that baseline DA competes effectively with [^{123}I]IBZM binding. Amphetamine reduction in V_3'' was significantly blunted in αMPT-pretreated animals: in baboon A, the 0.3 mg/kg dose induced a 12% decrease in V_3'' compared to 25% in control conditions, whereas in baboon D the 0.5mg/kg dose induced a 5% displacement instead of 23% in control conditions. Thus, the amphetamine effect was attenuated by an average of 66% of control values by αMPT pretreatment, confirming the role of DA release in amphetamine-induced reduction in [^{123}I]IBZM binding.

Low-dose raclopride experiments

To better understand the long-lasting effect of amphetamine injection on tracer specific binding (>4 h), we assessed the kinetic of [^{123}I]IBZM displacement after injection of subsaturating doses of raclopride (n = 2) in baboon E. The PET literature indicates that, in humans, i.v. injection of raclopride at a dose of 8 μg/kg provides between 50 and 100% receptor occupancy within 20 min and that raclopride has washed out by approximately 2 h postinjection (Farde et al., 1990; Hietala et al., 1994). During [^{123}I]IBZM constant infusion, raclopride was injected at 180 min as an i.v. bolus at doses of 4 and 8 μg/kg, and the data were collected for 4 h after the raclopride injection. The kinetics of raclopride-induced [^{123}I]IBZM displacement was similar to the kinetics of amphetamine-induced [^{123}I]IBZM

TABLE II. Decrease V3″ during [123I]IBZM constant infusion after amphetamine challenge test[1]

	Baboon A		Baboon B		Baboon D	
Challenge	Baseline V_3''	Decrease V_3'' (%)	Baseline V_3''	Decrease V_3'' (%)	Baseline V_3''	Decrease V_3'' (%)
Control	0.68	2	0.79	3	—	—
Amphetamine 0.3 mg/kg	0.66	25	0.74	21	0.73	15
Amphetamine 0.5 mg/kg	0.63	37	0.80	24	0.82	23
Amphetamine 1.0 mg/kg	0.58	47	0.77	42	0.84	26

[1] V_3'' is the specific to nonspecific partition coefficient at equlibrium and is calculated as (striatum-occipital)/occipital activities at equilibrium. Baseline V_3'' was measured from 180–240 min. Amphetamine was injected at 240 min. Postamphetamine V_3'' was measured from 360–420 min. Change in V_3'' is expressed as % of baseline V_3''. In contrast with [123I]IBF experiments (Table I), we observed a significant dose effect with [123I]IBZM in this dose range (two-way ANOVA with dose and animal as factors; dose P = 0.016; animal: P = 0.029) (Fig. 6).

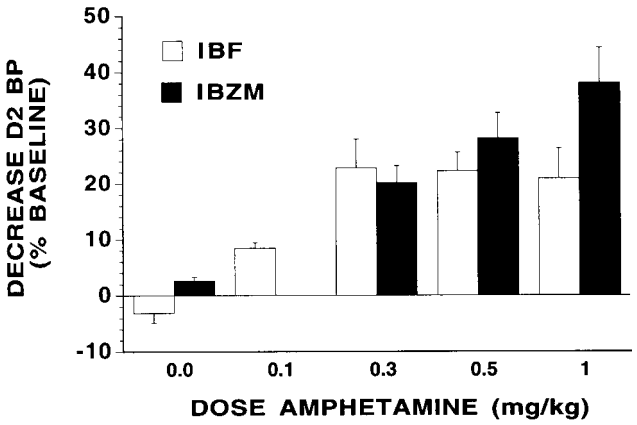

Fig. 6. Dose-response curve of amphetamine on [123I]IBF and [123I]IBZM V_3''. Each bar represents the mean ± S.D. of three experiments performed in three different baboons. Experiments at 0.1mg/kg were performed only with [123I]IBF. Dose factor was estimated with two-way ANOVA with doses and baboons as cofactors. In the 0.3–1.0 dose range, there was no amphetamine dose effect on [123I]IBF BP decrease ($P = 0.89$), but there was a significant dose effect on [123I]IBZM BP decrease ($P = 0.029$).

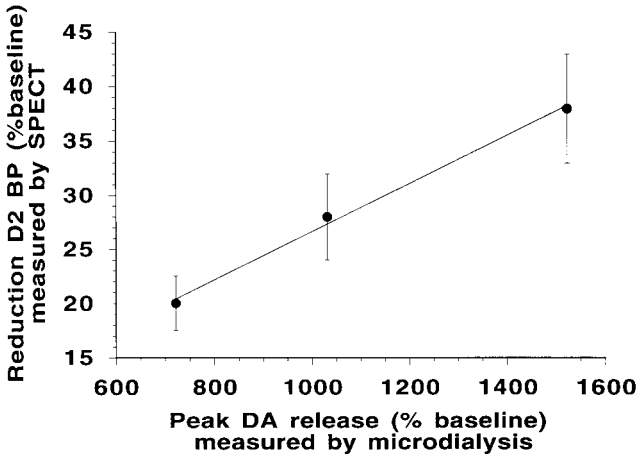

Fig. 7. Correlation between amphetamine-induced peak DA release as measured with microdialysis (x axis) and the decrease in [123I]IBZM D_2 V_3'' as measured with SPECT (y axis). Microdialysis measurements (n = 1 per dose) were obtained in one vervet monkey. Each SPECT measurement is the mean ± SD of three experiments per dose performed in three baboons. Although these measures were not obtained in the same species, they suggest that the SPECT measurements show a dose-response relationship compatible with microdialysis measurements, supporting the use of this paradigm as a noninvasive assessment of amphetamine-induced DA release.

displacement: [123I]IBZM specific binding decreased slowly and stabilized at a lower level for at least 4 h after the raclopride injection (Fig. 8). Comparing V_3'' measured before raclopride injection (120–180 min) to V_3'' measured from 2–4 h after raclopride injection revealed a 19% and 32% reduction in V_3'' after 4 μg/kg and 8 μg/kg doses, respectively.

DISCUSSION

Experiments presented in this paper showed that 1) amphetamine i.v. injections in the range of 0.03–1.5 mg/kg induce a dose-dependent increase in extracellular DA concentration as measured with microdialysis, 2) acute αMPT pretreatment significantly reduces baseline and postamphetamine extracellular DA concentration, 3) amphetamine induces a prolonged reduction of [123I]IBZM and [123I]IBF in vivo binding to D_2 receptor as measured with SPECT, 4) [123I]IBZM binding is more affected than [123I]IBF binding by high doses of amphetamine (>0.3 mg/kg), 5) [123I]IBZM (but not [123I]IBF) displacements following various doses of amphetamine (0.3–1 mg/kg) correlate well with peak DA release measured with microdialysis, 6) the effect of amphetamine on D_2 BP can be measured with SPECT with excellent reproducibility, 7) this effect is significantly blunted by αMPT pretreatment, and 8) the kinetics of amphetamine-induced [123I]IBZM displacement are similar to the kinetics of [123I]IBZM displacement after subsaturating doses of raclopride. Together, these experiments support the validity of SPECT measurement of amphetamine-induced DA release with [123I]IBZM.

In our previous work, we reported that the washout rate of [123I]IBZM from baboon striatum was increased by amphetamine (Innis et al., 1992). However, the washout rate of a tracer after single bolus injection is affected not only by the receptor affinity and density but also by the regional cerebral blood flow and the clearance of the tracer from the plasma. Consequently, the amphetamine effect on the [123I]IBZM washout rate might have been due to increased endogenous competition, changes in striatal blood flow, increased [123I]IBZM plasma clearance, or any combination of these factors. In contrast, under sustained equilibrium conditions, the activity ratio between brain regions (V_3'') is not

TABLE III. Reproducibility of amphetamine induced decrease in D_2 V_3''

Baboon	Tracer	Amphetamine dose (mg/kg)	Delay (days)	Decrease V_3'' (%)		
				Test	Retest	Difference
B	[123I]IBF	0.3	158	11	11	0
B	[123I]IBF	0.5	33	16	13	3
C	[123I]IBZM	1.0	64	26	28	2

TABLE IV. Effect of alpha-methyl-para-tyrosine (αMPT) pretreatment[1]

Baboon	Amphetamine dose (mg/kg)	Baseline V_3''			Amphetamine-induced decrease V_3'' (%)		
		Control conditions[1]	αMPT pretreated	Change (%)	Control conditions[1]	αMPT pretreated	Change
A	0.3	0.66	0.85	+28	25	12	−52
D	0.5	0.82	1.07	+30	23	5	−79

[1]Control condition = absence of αMPT pretreatment.

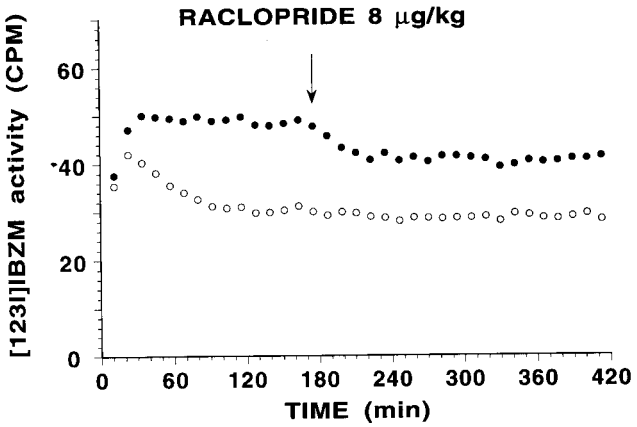

Fig. 8. Striatal (closed circles) and occipital (open circles) activities during [123I]IBZM bolus plus constant infusion in baboon E. Raclopride (8 μg/kg) was injected as an i.v. bolus at 180 min (arrow). Despite the fact that raclopride washes out rapidly from the receptor, [123I]IBZM specific binding was still reduced at 4 h postraclopride experiment.

affected by regional blood flow or peripheral metabolism (Carson et al., 1992; Laruelle et al., 1994a). Therefore, amphetamine-induced changes in V_3'' during tracer constant infusion only reflects changes in D_2 receptor parameters, and this measure is not contaminated by "peripheral" effects. This superior method was thus chosen to investigate the validity of this paradigm as a noninvasive measure of DA release.

Since this method is proposed as a measure of the magnitude of DA release, it was important to investigate the relationship between amphetamine-induced DA release and tracer displacement. The reduction in [11C]raclopride binding produced by GBR-12909 in baboons or methylphenidate in humans was not increased with increasing doses of the pharmacological agent (Dewey et al., 1993; Volkow et al., 1994). These observations raised concerns about the validity of these measurements as an indicator of DA release. However, the lack of microdialysis data impaired the interpretation of these results, given that the difference in DA release following high and low doses of these pharmacological agents was not documented. While the dose-response relationship of amphetamine-induced DA release has been studied in the rat (Kuczenski and Segal, 1989), to our knowledge no data were available in the literature that documented the dose-response relationship of amphetamine-induced DA release in the primate with microdialysis. Therefore, this information was obtained in order to evaluate the SPECT data.

Microdialysis data indicate that amphetamine enhances DA release in the vervet caudate in a dose-dependent manner in the 0.03–1.5 mg/kg dose range. This observation should be viewed with caution because only one animal was used in the dose-response curve. However, this observation was comparable to the dose-dependent increase in DA release produced by amphetamine in rodents (Florin et al., 1995; Kuczenski and Segal, 1989). In this dose range, we observed, with SPECT, a similar dose-effect relationship when experiments were performed with [123I]IBZM (but not with [123I]IBF). These data indicated that [123I]IBZM is probably a more appropriate tracer than [123I]IBF for noninvasive measurement of amphetamine-induced DA release. Despite the limitations of this comparison (microdialysis and SPECT experiments were conducted in different nonhuman primate species, and only one animal was studied with microdialysis), the relationship between microdialysis and SPECT measurements after various doses of amphetamine was linear, which is optimal for the use of this SPECT paradigm as a measure of DA release. These data should be regarded as preliminary. Simultaneous measurement of DA release by microdialysis and SPECT are needed to provide better characterization of the relationship between these measurements.

In contrast, [123I]IBF binding appeared to be less affected than [123I]IBZM by changes in endogenous DA concentration after high doses of amphetamine (>0.3 mg/kg). Previous studies have documented differences between PET radiotracers in vulnerability to endog-

enous competition. Two studies performed in rodents reported that the in vivo binding of the high affinity butyrophenone N-methylspiperone (K_D = 0.1 nM) was less affected by endogenous competition than that of the comparatively lower affinity benzamide, raclopride (K_D = 1 nM) (Seeman et al., 1989; Young et al., 1991). [^{123}I]IBZM affinity for D_2 receptor being lower (0.4 nM) than [^{123}I]IBF (0.1 nM), the results of this study are consistent with a role of tracer affinity in the susceptibility to endogenous competition in the context of acute pharmacological challenges. We have previously shown that the in vivo uptake of [^{123}I]epidepride, another iodinated benzamide with very high affinity for D_2 receptors (K_D = 0.05 nM), is not affected at all by amphetamine injection (Al-Tikriti et al., 1994).

While it might seem intuitively reasonable to postulate that tracer affinity might play a role in vulnerability to endogenous competition, it should be kept in mind that, at equilibrium, the ability of a competitor (such as DA) to displace a radiotracer present at tracer dose is independent of the affinity of the radiotracer (Cheng and Prusoff, 1973). In vitro, the IC_{50} of DA to displace tracer amounts of [^{125}I]IBZM and [^{125}I]IBF from D_2 receptors is similar (unpublished results). Therefore, these results imply that the Cheng and Pursoff equation (Cheng and Prusoff, 1973) does not apply to this in vivo situation, probably because equilibrium conditions are violated due to the constantly changing level of intrasynaptic DA after pharmacological challenge.

Logan et al. (1991) and Kessler et al. (1993a) proposed that lipophilicity is another property that influences the vulnerability of a radiotracer to enogenous competition. [^{123}I]IBZM being more lipophilic than [^{123}I]IBF, these results support this contention as well. At low receptor occupancy, the effective off rate of a radiotracer from the receptor compartment depends not only on the rate of dissociation from the receptor (k_{off}) but also on the ability of this radiotracer to cross the synaptic membranes, which is enhanced by lipophilicity (Frost and Wagner, 1984). Kinetic analysis of [^{123}I]IBF and [^{123}I]IBZM single bolus experiments in baboons revealed that the kinetic parameter k_4, which measures the effective rate of escape from the receptor compartment at negligible receptor occupancy, is about three times slower for [^{123}I]IBF (0.0128 $\pm$ 0.0018 min^{-1}, n = 3) (Laruelle et al., 1994b) than [^{123}I]IBZM (0.0364 $\pm$ 0.0054 min^{-1}, n = 2) (unpublished results). Thus, both a relatively modest affinity and a relatively high lipophilicity contribute to a rapid escape of the tracer from the receptor compartement and to an increased vulnerability to fluctuating levels of endogenous competitor. However, we cannot rule out other explanations for the differences between the [^{123}I]IBZM and [^{123}I]IBF vulnerability to endogenous competition, such as binding to different motifs or configuration of the D_2 receptors.

The range of amphetamine-induced decrease in [^{123}I]IBZM binding observed in this study is similar to the range of amphetamine-induced decrease in [^{11}C]raclopride binding reported by Dewey et al. (1993) in the anesthetized baboon. The relatively small magnitude of these changes (<40%) contrasts with the robust increase in extracellular DA measured by microdialysis and might be explained by two factors. 1) D_2 receptors are configured in states of high or low affinity for DA, with approximately 50% of the receptors contributing to each state (Seeman and Grigoriadis, 1987; Sibley et al., 1982). [^{123}I]IBZM, being an antagonist, binds with equal affinity to both states. Thus, it is possible that endogenous DA does not compete efficiently with [^{123}I]IBZM binding to D_2 receptors that are in the low affinity configuration, leaving only about 50% of [^{123}I]IBZM binding susceptible to endogenous competition. 2) A substantial number of D_2 receptors could be internalized within the neurons. [^{123}I]IBZM, being lipophilic, would bind to internalized receptors as well as receptors externalized in the cellular membrane. In contrast, DA would not access internalized receptors, and these would constitute a pool of receptors not affected by endogenous competition. Lack of knowledge about the in vivo proportion of high vs. low affinity configurations or internalized vs. externalized location of D_2 receptors precludes more informed evaluation of these explanations.

Experiments performed with αMPT confirmed that the amphetamine effect on [^{123}I]IBZM binding is mediated by DA release. αMPT is a specific competitive inhibitor of tyrosine hydroxylase (K_i = 17 μM), an enzyme essential to DA synthesis (Spector et al., 1965). Therefore, the tissue level of αMPT determines the degree of enzymatic inhibition, and the duration for which this tissue level is maintained determines the magnitude of DA depletion. Continuous infusion of αMPT appeared as an adequate mode of administration to provide a sustained αMPT level in a controlled condition. The αMPT dose used in this study was extrapolated from pharmacokinetic studies in humans (Engelman et al., 1968) to provide near complete synthesis inhibition. This regimen induced a reduction of about 50% of basal DA concentration after 4 h. Interestingly, [^{123}I]IBZM V_3'' was increased by an average of 29% in the baboons after αMPT infusion as compared to the control condition. This increase exceeded the test-retest reproducibility for measurement of D_2 V_3'' in baboons (coefficient of variation of 6%) (Table 2). These data suggest that a significant proportion of D_2 receptors is occupied by DA at baseline level and that DA depletion could contribute to the increased [^{123}I]IBZM binding observed in the contralateral side of patients with asymmetric Parkinson's disease (Kinable et al., 1995; Laulumaa et al., 1993).

Microdialysis experiments in the rat have demonstrated that αMPT pretreatment significantly reduces

amphetamine-induced DA release (Butcher et al., 1988; Nash and Yamamoto, 1993). Studies presented in this paper showed that the amphetamine-induced increase in DA was significantly reduced by αMPT pretreatment. Similarly, the amphetamine effect on [^{123}I]IBZM BP was significantly blunted. These experiments confirm that amphetamine-induced decrease in [^{123}I]IBZM binding is mediated by endogenous DA release.

A significant time interval was observed between the peak of extracellular DA release (which occurred within 20 min of amphetamine injection) and the achievement of the tracer displacement (90–120 min after amphetamine injection). This phenomenon might be due to the slow in vivo receptor off rate of high affinity tracers at negligible receptor occupancy (Frost and Wagner, 1984; Laruelle et al., 1994a,b; Perry et al., 1980). More enigmatic was the observation that the D_2 BP is still reduced up to 240 min after amphetamine injection. In none of the 27 experiments performed with amphetamine did we observe a return of D_2 BP to preamphetamine levels nor even a trend toward an increase. In contrast, microdialysis experiments indicated that extracellular DA concentration had dropped to 34% of peak value at 120 min postamphetamine injection.

Two hypotheses were initially formulated to explain this phenomenon. 1) The time discrepancy between microdialysis and SPECT measurements could reflect differences in extrasynaptic and intrasynaptic DA concentration (see discussion in Grace, 1991). Behavioral effects of amphetamine last longer than the increase in extracellular DA concentration recorded by microdialysis (Kuczenski and Segal, 1989). Microdialysis might measure the initial overflow of DA into the extracellular fluid (due to increased DA release following amphetamine) but might be less sensitive to sustained increased intrasynaptic DA release due to other properties of amphetamine such as DA synthesis stimulation and DA metabolism inhibition (Pearl and Seiden, 1979; Schwarz et al., 1980). If this hypothesis is correct, the prolonged decrease in D_2 BP induced by amphetamine would reflect a sustained increase in intrasynaptic DA concentration that would not be recorded by microdialysis. 2) An alternate hypothesis was suggested by the fact that exposure of D_2 receptors to high levels of agonist stimulation induces rapid receptor downregulation in cell cultures (Barton et al., 1991). In this case, the observed effect would be the end result of the combination of transmitter release and adaptative changes of receptors.

The experiments performed at subsaturating doses of raclopride showed that none of these hypotheses were needed to explain the prolonged state of reduced [^{123}I]IBZM binding observed after amphetamine injection. Raclopride was selected because of its fast binding kinetic as documented in PET experiments with [^{11}C]raclopride (Farde et al., 1990; Hietala et al., 1994). The raclopride binding kinetic approximates the kinetic of amphetamine-induced DA release as measured with microdialysis. Furthermore, raclopride, being a D_2 antagonist, is unlikely to cause D_2 receptor downregulation. Despite this, we observed that [^{123}I]IBZM levels remained reduced up to 4 h after raclopride injection. The absence of return to baseline binding during the time frame of these experiments is likely due to the slow kinetics of in vivo association under near equilibrium conditions (Malison et al., in press). Additional experiments during which the concentration of both the tracer and the competitor is measured will be needed to develop a model of the slow kinetics of [^{123}I]IBZM dissociation and association observed in these experiments.

In conclusion, these studies demonstrated that the reduction in [^{123}I]IBZM BP following amphetamine is not due to peripheral effects of the drug, is related to the magnitude of DA release, and is blunted by DA synthesis inhibition. Together, these experiments validate the use of this paradigm to assess amphetamine-induced DA release in humans (Laruelle et al., 1995). This method will offer a unique opportunity to investigate dopaminergic system function in neuropsychiatric illnesses.

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