J Neural Transm (1997) 104:329–339

Journal of Neural Transmission

Amphetamine effects on dopamine release and synthesis rate studied in the Rhesus monkey brain by positron emission tomography

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Accepted February 24, 1997

Summary. Positron emission tomography (PET) was used in a multitracer protocol to evaluate D-amphetamine induced effects on dopamine biosynthesis rate and release in propofol anesthetized Rhesus monkeys. L-[β-^{11}C]DOPA was used as biochemical probe to study the brain dopamine biosynthesis rate whilst dopamine release was followed by the binding displacement of the [^{11}C]-radiolabelled dopamine receptor antagonists, raclopride and N-methylspiperone. Studies were performed with either a constant rate intravenous infusion of D-amphetamine aiming at plasma concentrations of 0.2 to 25 ng/ml or with intravenous bolus doses of 0.1 and 0.4 mg/kg. Decreased binding of the dopamine receptor antagonists was measured in both modes of D-amphetamine administration but notably [^{11}C]N-methylspiperone was less able to sense D-amphetamine induced release of dopamine. At plasma concentrations aimed above 1 ng/ml a levelling off of the binding of [^{11}C]raclopride at 68 ± 8.1% of the baseline value indicated that displacement was only possible from a fraction of the binding sites. Amphetamine was observed to increase the rate constant for L-[β-^{11}C]DOPA utilization in the brain. This was most likely due to an acutely induced subsensitivity of presynaptic dopamine receptors. L-[β-^{11}C]DOPA and [^{11}C]raclopride were found suitable to indicate changes in dopamine synthesis rate and release respectively using PET and can be used to mirror drug-induced changes of brain dopaminergic function.

Keywords: Amphetamine, [^{11}C], L-DOPA, raclopride, N-methylspiperone, CBF, PET, monkeys.

Introduction

Simultaneous quantitation of neurotransmitter release and biosynthesis rate is fundamental in the characterization of disease processes and in the assessment of the effects of drug treatment. Positron emission tomography (PET) combined with multitracer protocols with selected positron emitting tracers has been used to characterize the dopaminergic function in a number of investigations. These include studies on Parkinson's disease (Tedroff et al., 1995; Torstenson et al., 1997) and following exposure to organic solvents (Edling et al., 1997). The selection of tracers comprised L-[β-^{11}C]DOPA which has been validated to quantify decarboxylation rate in the biosynthesis of dopamine (Tedroff et al., 1992; Lindner et al., 1995), [^{11}C]-nomifensin to measure the number of dopamine terminals and various dopamine receptor antagonists to quantitate the number of available dopamine receptors.

D-amphetamine is a potent releaser of monoamines (McMillen, 1983) and has been utilized to sense dopamine release to the synaptic cleft as a decreased binding of dopaminergic antagonists in the brain of rats and monkeys (Seeman et al., 1989; Young et al., 1991; Dewey et al., 1991, 1993; Innis et al., 1992). In PET studies using the two dopamine selective receptor antagonists, [^{11}C]raclopride (Dewey et al., 1993) and [^{18}F]N-methylspiperone (Dewey et al., 1991), their decreased binding has been used as a quantitative measure of dopamine release in the monkey brain. A similar quantitation of the selective binding of [^{123}I]-iodinated benzamide has been performed with single photon emission computed tomography (Innis et al., 1992; Laurelle et al., 1997). Studies on D-amphetamine induced displacement from other subtypes of dopamine receptors have not been published so far.

Dewey et al. (1993) have shown that the decrease of [^{11}C]raclopride binding following a D-amphetamine bolus dose to be more consistent than that observed with [^{18}F]N-methylspiperone. From similar observations in the rat this difference has been suggested to be related to the higher affinity and slower kinetics of N-methylspiperone (Young et al., 1991). For an understanding of the brain dopaminergic function changes in dopamine release studies on effects on dopamine synthesis rate should also be followed. The latter may be measured as the radioactivity cumulation in the striatal region after administration L-[β-^{11}C]DOPA (Tedroff et al., 1992; Hartvig et al., 1991). In the present study the effects of D-amphetamine on the release and biosynthesis of dopamine in the striatum of the Rhesus monkey were quantified. The release of dopamine from nerve terminals was measured as the displacement of radioactivity of the two D_2-dopamine receptor antagonists, [^{11}C]raclopride and [^{11}C]N-methylspiperone, and the dopamine biosynthesis rate was assessed using L-[β-^{11}C]DOPA.

Material and methods

Animals

Twelve female Rhesus monkeys (Macaca Mulatta) 20 to 30 years of age and weighing 8 to 11 kgs from the Primate Research Laboratory of Uppsala University PET Centre were

used after an overnight fast. Before the study, the monkeys had been housed in groups of four to eight for at least four months and no monkey had been exposed to dopaminergic drugs for at least three years prior to the study. Anesthesia was induced with 100 mg of intramuscular ketamine (Ketalar®, Parke Davis). After transportation to the investigation area of the Uppsala University PET Centre one arterial line was inserted in arteria femoralis in studies of cerebral blood flow. A venous catheter was inserted in each hind leg of the monkey, one for administration of radioactive tracer and the other for collection of venous blood. After tracheal intubation, muscle relaxation was achieved with intravenous atracurium, 0.5 mg/kg/h (Tracrium®, Wellcome). Ventilation was performed with 30% oxygen in air. Anesthesia was continued with propofol (Diprivan®, ICI) starting with 10–12 mg/kg/h for 15 min. and continued throughout with a maintenance infusion of 8 to 12 mg/kg/h. The studies were approved by the Animals Ethics Committee of the Uppsala University (permission C03/92 and C289/94).

A stabilization period of at least 30 min. was used before radiotracer administration throughout. In eight monkeys baseline PET scans with [^{11}C]raclopride and [^{11}C]N-methylspiperone were performed before the start of continuos infusion of D-amphetamine aiming at plasma concentrations of 0.2 to 25 ng/ml in the monkeys. D-amphetamine was given as bolus dose to achieve the desired plasma concentration based on volume of distribution which immediately was followed by constant rate infusion equal to an estimated plasma clearance of D-amphetamine. Dose rates of D-amphetamine were calculated from a volume of distribution of 5 L/kg and a plasma clearance of 0.7 L/kg × h (Dollery et al., 1991). In two other monkeys, baseline PET scans of [^{11}C]raclopride were performed followed by measurement of cerebral blood flow (CBF) using [^{15}O]-water. Baseline [^{11}C]N-methylspiperone PET-studies immediately followed measurement of CBF. This sequence of PET scans was repeated after slow intravenous administration 0.4 mg/kg D-amphetamine, each time 15 min. before the investigation with PET.

Baseline PET scans were also performed with L-[β-^{11}C]DOPA in two monkeys. D-amphetamine was then given as slow intravenous bolus doses of 0.4 mg/kg 15 min. before the second PET scan with L-[β-^{11}C]DOPA. In two other monkeys the stability of the effects of D-amphetamine infusion aiming at plasma concentrations of 1 and 10 ng/ml were studied. PET scans with L-[β-^{11}C]DOPA were performed before and then 1, 4 and 5 hours after start of infusion.

Radiochemical synthesis

[^{11}C]Carbon dioxide was produced by the ^{14}N(p,α)^{11}C reaction using a nitrogen gas target and 17 MeV protons produced by the MC17 cyclotron (Scanditronix, Uppsala, Sweden) at the Uppsala University PET Centre. L-[β-^{11}C]DOPA was synthetized using a combination of organic synthesis and multienzymatic processes (Bjurling et al., 1990). [^{11}C]Raclopride and [^{11}C]N-methylspiperone were obtained after conversion of [^{11}C]carbon dioxide to [^{11}C]methyl iodide which was used in the N-alkylation of the corresponding desmethyl compounds (Långström et al., 1987). The reaction products were purified by liquid chromatography and the appropriate fraction collected, evaporated and the residue dissolved in sterile buffer. The final solution was filtered through a 0.22 μM filter. Identity, chemical and radiochemical purity were determined by liquid chromatography and in comparison with the retention of the appropriate reference compound. The radiochemical purity was always higher than 95%. The specific radioactivity of the radiotracers were 5–10 GBq/μmol. The radioactive dose of the tracers varied 80 to 200 MBq depending on the used PET camera system.

Positron emission tomography

Studies on radioactivity distribution in the monkey brain were performed using either of the two positron emission tomographs; GE 2048-15B Plus and GE4096-15WB-Plus

(General Electric Medical Systems, Uppsala, Sweden). The radioactivity was measured in 15 transaxial slices interspaced with 6.8 mm. The inplane spatial resolution at full width half maximum of the camera systems was 5 and 6 mm, respectively. The images were reconstructed for data collected from each time frame, using a contour finding algorithm for attenuation correction and filtered with a 4.2 mm Hanning filter.

To prevent movements, the head of each monkey was gently fixed in position using plugs attached to the auditory passage. The monkey remained in the tomograph between scans to minimize changes in head position from one scan to the next. Vital signs including blood pressure, heart and respiratory rate and body temperature were monitored throughout. In all studies, recovery from anesthesia was rapid without sequelae.

The radioactive dose was injected in a venous catheter in one hind leg of the monkey and the tomograph was started. Images were recorded for time frames of fifteen times 60 seconds followed by fifteen 200 seconds frames. PET continued for 60 minutes. The radioactivity was corrected for physical decay from the time of radiotracer administration. Regions of interest (ROI) in the brain were drawn from reconstructed summation images and in comparison with a Rhesus monkey brain atlas made after cryosectioning. The two striata were delineated as circles of 0.9 cm^2 each. A reference tissue ROI was drawn in a large region of non-dopaminergic tissue situated posterior and in the same brain slice as the striata (about 8 cm^2). The cerebellum was delineated and in some cases also the frontal, temporal and occipital cortices (0.5 cm wide) in comparison with the brain atlas. ROIs were delineated in the baseline PET scans and then copied to the corresponding slice in the subsequent PET scans. A reslicing procedure was applied to ensure identical positioning of the monkey before analysis, whenever movements within the gantry were evident (Andersson, 1995).

Cerebral blood flow measurements were performed using bolus injections of ^{15}O-radiolabelled water. PET scanning started immediately following injection of approximately 500 MBq of $H_2{}^{15}O$ in saline. Blood radioactivity was sampled with a frequency of 1 Hz by an automatic device (Eriksson et al., 1988) calibrated to yield radioactivity concentration measurements.

Calculations

For calculation of specific binding of [^{11}C]raclopride and [^{11}C]N-methylspiperone, the following assumptions were made: 1) The reference area did not contain any specific receptors for binding, 2) only a minute fraction of available binding sites were occupied, 3) radiolabelled metabolites did not form or penetrated poorly the blood brain barrier and 4) the dissociation in the case of [^{11}C]N-methylspiperone from the binding sites during the investigation period was negligible.

The mathematical description of the change in radioactivity over time for [^{11}C]N-methylspiperone was done using a reduced compartment model comprising a compartment for radioactivity in the extracellular space and nonspecifically bound in the brain and one compartment for specific utilization of radioactive tracer. Since both compartments have blood as input function, the blood compartment was reduced. The method used the reference tissue, i.e. back of the brain, in the same slice as input function and effectively linearized striatal [^{11}C]N-methylspiperone uptake. The rate k_3, including the product of the binding association rate of the tracer and the number of specific binding sites, was determined by linear regression analysis using least square weighted data collected 5 to 60 minutes of real time after tracer injection. To calculate the weight factor for each point in the linear regression analysis an empirical formula was used for calculation of the standard deviation for a given pixel (Carson et al., 1986).

The binding of [^{11}C]raclopride was calculated from equilibration of the striatum to cerebellum radioactivity ratio, using mean data from consecutive time frames 20 to 50 minutes after tracer injection. The equilibrium striatum to cerebellum uptake ratio can be related to striatal dopamine D_2-receptor density since the number of D_2 receptors in the

cerebellum are insignificant (Brooks et al., 1992). The binding potential was defined as the striatum to cerebellum uptake ratio minus one. This represents the ratio of specific to unspecific and tissue free [^{11}C]raclopride, which gives a measure of available binding sites (Tedroff et al., 1995). Specific striatal binding of L-[β-^{11}C]DOPA was described by the same mathematical model as for the calculation of [^{11}C]N-methylspiperone. The slope of the regression line is proportional to the rate constant for the unidirectional flux into the compartment for specific utilization and is dependent on the activity of the decarboxylase enzyme and is shown to represent the rate for transport and conversion of the tracer to [^{11}C]dopamine within the region of interest (Lindner et al., 1995).

Cerebral blood flow (CBF) was calculated using an autoradiographic technique described previously (Hersovitch et al., 1983; Raichle et al., 1983) corrected for delay and dispersion (Meyer, 1989). An integration time of 45 seconds was used and the distribution volume was assumed to be 0.95 ml/ml.

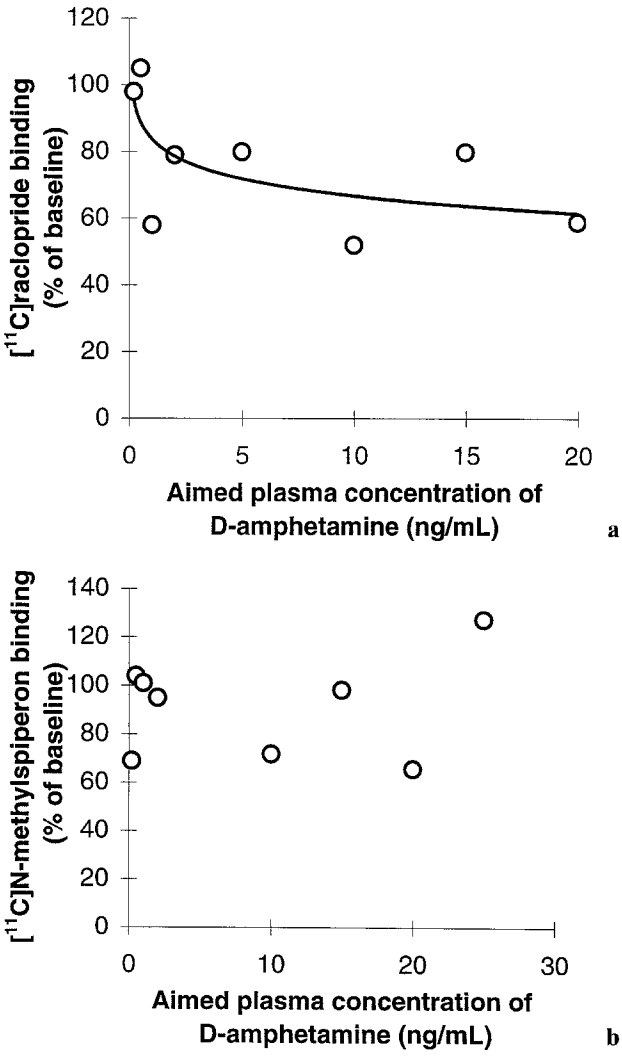

Fig. 1. Change of dopamine receptor binding at different plasma concentrations of D-amphetamine as evaluated with [^{11}C]-raclopride (**a**) and [^{11}C]N-methylspiperone (**b**), respectively

Results

Effect on binding of [^{11}C]raclopride and [^{11}C]N-methylspiperone

When performing D-amphetamine infusion aiming at plasma concentrations in the range 0.2 to 25 ng/ml a decreased [^{11}C]raclopride binding was observed. The results from all monkeys are shown in Fig. 1a. The changes were larger than the intraindividual variation of ±5% in raclopride binding potential measured in test-retest investigations. The interindividual variation in binding during baseline scans was large with a relative standard deviation of striatal binding potential in eight monkeys of 25%. Above an aimed plasma concentrations of 1 ng/ml, the decrease in binding potential levelled off at about 75 to 60% of baseline values. Binding decreased by a mean of 31.2 ± 8.1% irrespective of dose.

[^{11}C]N-methylspiperone was not similarly sensitive to indicate changes induced by D-amphetamine infusion in the striatum and even less in cortical areas of the brain (data from cortical areas not shown). Intra- and interindividual variations in test-retest analysis of k_3 in the striatum were 7 and 33%, respectively during baseline. No consistent effect on radioactivity displacement of [^{11}C]N-methylspiperone was seen and in some experiments the k_3 values even increased during D-amphetamine infusion (Fig. 1b). The mean decrease of k_3 was 6.0 ± 17.5% of baseline values.

A lower binding of both [^{11}C]raclopride and [^{11}C]N-methylspiperone was measured in the striatal region following an intravenous bolus dose of 0.4 mg/kg of D-amphetamine. The decrease was more prominent using

Table 1. Effect of amphetamine on decarboxylation rate in striatum of the monkey

A. Effect of bolus doses

Dose of amphetamine	Decarboxylation rate min^{-1}	Correlation coefficient
0 mg/kg	0.0156	0.97
0 mg/kg	0.0148	0.96
0.1 mg/kg	0.0163	0.97
0.1 mg/kg	0.0157	0.98
0.4 mg/kg	0.0178	0.98
0.4 mg/kg	0.0161	0.97

B. Effect of continous infusion

Time (hour) from start of infusion	Decarboxylation rate, min^{-1} Aimed plasma concentration, ng/ml	
	1 ng/ml	10 ng/ml
0	0.0132	0.0144
1	0.0137	0.0138
4	0.0154	n.d.
5	0.0148	0.0136

[^{11}C]raclopride as radiotracer (mean decrease 43% in two monkeys) as compared with [^{11}C]N-methylspiperone (mean decrease 14%).

Effect on decarboxylation rate of L-[β-^{11}C]DOPA

Amphetamine in bolus doses of 0.1 and 0.4 mg/kg slightly but dose-dependently increased the k_3 value i.e. the decarboxylation rate of L-[β-^{11}C]DOPA (Table 1a). This increase was larger than the intraindividual variation (±3%) of the rate measured in test-retest studies on L-[β-^{11}C]DOPA decarboxylation rate in the Rhesus monkey brain as previously reported by Tedroff et al. (1996). Infusion of D-amphetamine in doses aiming at steady state plasma concentrations of 1 and 10 ng/ml resulted in no change of the k_3-value shortly after start of infusion. After 4 hours and 5 hours a higher k_3-value was seen in one of the two monkeys studied (Table 1b).

Cerebral blood flow

Global CBF decreased by 50% following a D-amphetamine bolus dose of 0.4 mg/kg. Regional decreases of the same magnitude in different cortical areas, in the striatum and thalamus were measured in the two Rhesus monkeys studied.

Discussion

Using positron emission tomography with the selected tracers it was possible to indicate some pharmacological effects of D-amphetamine such as altered dopamine release and biosynthesis rate. The binding potential of [^{11}C]raclopride in the striatum was profoundly decreased following a D-amphetamine bolus dose but the decrease of binding of N-methylspiperone was less pronounced. A more reliable and constant D-amphetamine effect should be obtained during constant rate infusion of the drug. Therefore a two rate dosage regimen was used to rapidly achieve steady state conditions in order to match the large volume of distribution and low clearance of D-amphetamine (Dollery et al., 1991). Similarly, the [^{11}C]raclopride binding potential decreased following increasing infusion doses of D-amphetamine whilst [^{11}C]N-methylspiperone binding showed large intra- and interindividual variations and was inconsistently sensitive to D-amphetamine induced effects. Taking into account previous reports of D-amphetamine dramatically increased striatal dopamine concentrations (Zetterström et al., 1983; Kuczenski and Segal, 1989) using the microdialysis technique in freely moving rats, the present results might be explained by a release of a newly synthetized pool of dopamine (Butcher et al., 1988) competing for postsynaptic binding with the dopamine receptor antagonists. The marked resistance of [^{11}C]N-methylspiperone to indicate synaptic release of dopamine is compatible with previous studies (Young et al., 1991; Dewey et al., 1991, 1993). The different behavior of these two tracers was previously interpreted as an effect of the higher receptor affinity of N-methylspiperone, and far higher than the affinity of dopamine itself (Young et al., 1991). Following infusion doses of D-amphetamine, the

binding potential levelled off at aimed plasma concentrations above 1 ng/ml. This observation that displacement became independent of D-amphetamine dose may indicate that a maximum release of the newly synthetized dopamine pool was reached or alternatively that binding occurred only to a subfraction of binding sites available for displacement, i.e. high affinity dopamine receptors. Thus, a full competition for raclopride binding sites was not possible. In vitro studies have shown that raclopride binds to multiple dopamine receptor subtypes but in striatum other binding sites apart from D_2-dopamine receptors were considered insignificant (Dewey et al., 1993). Similarly, N-methylspiperone only binds to other receptor populations with considerably lower affinities and therefore this binding is considered minor (Young et al., 1991). Therefore, it is concluded that displacement mainly occurred at D_2-dopamine receptors. The binding affinity of D-amphetamine to D_2-dopamine receptors is low (Burt et al., 1976) so competition for binding with the two radiotracers is therefore not likely. D-amphetamine has an action to inhibit the re-uptake of monoamines from the synaptic cleft (McMillen, 1983) whereas the binding of the two radiotracers to presynaptic reuptake sites is insignificant (Tedroff et al., 1992). The re-uptake transporter balances the synaptic neurotransmitter content (Leshner, 1996). It has been shown in knock-out mice that a lack of functional dopamine transporters resulted in no biochemical or behavioral response to D-amphetamine (Giros et al., 1996).

A D-amphetamine induced change of CBF may also affect the measured binding parameter. Following a bolous dose of 0.4 mg/kg of D-amphetamine a decrease in CBF of about 50% was observed in different brain areas in the two monkeys studied. The decreased binding occurred simultaneously with a decrease of CBF. However, it has been shown that raclopride (Logan et al., 1993) and N-methylspiperone (Dewey et al., 1991) binding are both insensitive to changes in CBF. Therefore, the postsynaptic receptor antagonist [^{11}C]raclopride, but not N-methylspiperone may be used with positron emission tomography to quantitate release of dopamine and can possibly be extended to patient studies to quantify drug effects on dopamine function in the brain.

An increase of decarboxylation rate of L-[β-^{11}C]DOPA was found following D-amphetamine bolus doses of 0.1 and 0.4 mg/kg. This increase was larger than test-retest variations of the tracer (Tedroff et al., 1997). A constant rate infusion of D-amphetamine initially had no consistent effect on decarboxylation rate but an increased rate was measured some 4 hours after start of infusion. Systemic D-amphetamine releases a newly synthetized pool of dopamine, a fraction of which is suggested to enter the storage pool, thus elevating the total concentration of dopamine (Miller and Shore, 1982). An severalfold increase of synaptic dopamine in combination with increased storage might in some animals result in an increased k_3-value. On the other hand D-amphetamine has a direct action on dopamine metabolism decreasing the content of dihydroxyphenyl acetic acid in the rat striatum (Argiolas et al., 1978) and thereby giving an apparent lower synthesis rate. However, selective blockade of enzymatic steps in dopamine turn-over has not changed the

k_3-value for decarboxylation rate measured in the Rhesus monkey (Tedroff et al., 1992). An explanation for the higher rate is an induced subsensitivity of presynaptic dopamine receptors occurring after D-amphetamine administration (Kamata and Rebec, 1984; White and Wang, 1984; Seutin et al., 1991). The subsensitivity is correlated with a higher firing rate and an increased number of active dopamine cells. The effects may occur acutely (Seutin et al., 1991) and might be of importance to explain an increased decarboxylation rate some hours after D-amphetamine infusion in the present study.

In conclusion, D-amphetamine induced a prominent cytosolic release of dopamine to the synaptic cleft which could be quantified by positron emission tomography with the dopamine receptor antagonist [^{11}C]-raclopride. [^{11}C]N-methylspiperone was not sensitive enough to detect these changes. The results support that effects of brain dopaminergic function of long-term D-amphetamine therapy can be studied with PET using a similar protocol using [^{11}C]-raclopride to indicate dopamine release and L-[β-^{11}C]DOPA to measure effects on decarboxylation rate.

Acknowledgements

Financial support was given by the Swedish Medical Research Council grant no 8645, which is gratefully acknowledged.

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Received September 4, 1996