Reproducibility of Repeated Measures of Carbon-11-Raclopride Binding in the Human Brain

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Carbon-11-raclopride has been successfully utilized with PET to assess changes in endogenous dopamine concentration after pharmacological intervention in the living baboon brain. For similar studies to be done in humans, measurements of ^{11}C-raclopride with no intervention need to be reproducible. In order to test the reproducibility (test-retest) of ^{11}C-raclopride binding in the human brain, we performed repeated studies on two different days. Studies were done in five normal controls with no pharmacological intervention. Time-activity (%dose/cc) curves for ^{11}C-raclopride in the basal ganglia (BG) and cerebellum (CBL) were highly reproducible with an average difference in peak uptake for repeated studies in the same individual of 4%. The BG to CBL ratio for the average activity concentration between 30 and 60 min showed differences that ranged from −7% to 8% between the repeated studies. Graphical analysis to obtain the distribution volume revealed intrasubject values that ranged from −9% to 7% for the ratio of the distribution volume in BG to that in CBL. These studies demonstrate that in order to use ^{11}C-raclopride to measure an individual's change in relative dopamine concentration secondary to pharmacological or behavioral intervention, a change in striatal ^{11}C-raclopride binding in excess of 10% is required.

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Carbon-11-raclopride, a substituted benzamide, has been used to measure D2 dopamine receptor properties in the human brain with PET (*1–3*). The relatively lower affinity of raclopride for the D2 dopamine receptor (Kd = 1.1 n*M*) than that of other dopamine tracers utilized with PET such as spiperone and N-methylspiperone (Kd = 0.08 n*M*) make it more sensitive to competition with endogenous dopamine (*4–7*). Studies in rodents have demonstrated that raclopride binding is increased by pretreatment with drugs that deplete dopamine and decreased by drugs that increase dopamine concentration (*6–9*). Because of its sensitivity to endogenous dopamine, ^{11}C-raclopride has been used with PET to assess relative changes in dopamine concentration in the living baboon brain (*10*). These studies monitored changes in synaptic dopamine concentration secondary to pharmacological interventions by observing changes in ^{11}C-raclopride binding. Similarly, SPECT studies with the iodinated benzamide [5-N-(1-ethyl-2-pyrrolidinyl]-methyl-2-hydroxy-3-iodo-6-methoxybenzamide (IBZM), (Kd = 0.43 n*M*) have demonstrated the displacement of IBZM by endogenous dopamine in the living nonhuman primate brain (*11*). The feasibility of measuring relative changes in dopamine concentration using ^{11}C-raclopride requires that the measurements of ^{11}C-raclopride binding be reproducible in the same subject. The stability of ^{11}C-raclopride binding has been demonstrated for the anesthetized baboon brain when scanned on the same day (*10*). The stability of ^{11}C-raclopride binding measured in the awake human brain has not been reported. This study assesses the stability of ^{11}C-raclopride measurements in human brain by performing repeated ^{11}C-raclopride scans on the same subject on separate days.

METHODS

Subjects

The subjects were five normal healthy male volunteers (ages 21–46 yr) who were screened for absence of medical, neurological or psychiatric disease. Care was taken to exclude subjects with a past or present history of alcohol or drug use (except caffeine). Urine toxicology tests were performed twice in the week the scans were performed to ensure absence of psychoactive drug use. Informed consent was obtained from the subjects following the guidelines of the Human Studies Review Committee at Brookhaven National Laboratory.

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TABLE 1
Mass of Raclopride Injected and Integrated Plasma Concentration of ^{11}C and ^{11}C-Raclopride per Subject in Both Studies

Subject	Study	Weight (lb)	RACL (μg)	Plasma Integral: Total activity 30 min	Plasma Integral: Total activity 60 min	Plasma Integral: Unchanged ^{11}C-RACL 30 min	Plasma Integral: Unchanged ^{11}C-RACL 60 min
rcs001	1	184	12.1	1317	1966	1101	1468
	2		4.5	1377	2016	1163	1572
rcs002	1	158	10.1	1601	2445	1315	1877
	2		8.7	1694	2598	1422	2033
rcs003	1	243	12.1	1369	2093	1258	1803
	2		7.3	1365	2091	1261	1796
rcs004	1	173	14.6	1232	1886	1061	1502
	2		12.5	1243	1908	1128	1633
rcs005	1	222	13.5	1232	1886	1123	1610
	2		6.6	1247	1913	1156	1616

Scan

PET studies were carried out with a whole-body, high-resolution positron emission tomograph (6 × 6 × 6.5 mm FWHM, 15 slices, Computer Technologies, Incorporated, 931). To ensure accurate repositioning of subjects in the PET camera for the repeated scans, an individually molded headholder was made for each subject. The head of the subject was then positioned in the gantry with the aid of two orthogonal laser lines, one of which was placed parallel to the canthomeatal line and the other parallel to the sagittal plane. This strategy allows accuracy for repositioning within 2 mm (*12*). A chin strap device was used to minimize movement of the head during the scan. Subjects were scanned twice 24 hr apart at the same time of day using 3.8–12.5 mCi of ^{11}C-raclopride (*13*) (specific activity 0.5–1.5 Ci/μM at EOB. Table 1 provides the mass of raclopride injected for each of the studies and the weight of each subject). Prior to ^{11}C-raclopride injection, transmission scans were obtained to correct for attenuation. In preparation for the study, subjects had two catheters implanted, one in an antecubital vein for tracer injection and the other in the radial artery for blood sampling. Arterial sampling was used to quantitate total ^{11}C and unchanged ^{11}C-raclopride in plasma. Arterial samples were obtained using an automated blood sampling device (Ole Dich, Denmark) every 2.5 sec for the first 2 min, then every min from 2–5 min and then at 10, 15, 20, 30, 45 and 60 min. After injection of ^{11}C-raclopride, a series of 20 emission scans were obtained from time of injection up to 60 min (scans were taken each min for the first 10 min and then 5-min scans were taken for the next 50 min).

Assay of ^{11}C-Raclopride in Plasma

For quantitation of ^{11}C-raclopride in plasma, the following procedure was used. Acetonitrile (0.5 cc) was added to each plasma sample and the mixture was sonicated and centrifuged. Unchanged raclopride concentrations were determined by high-pressure liquid chromatography (HPLC) of a supernatant which had been previously spiked with unlabeled raclopride. The HPLC system consisted of a Waters Nova-Pak C_{18} column (3.9 × 300 mm) and a mobile phase of CH_3CN:0.01 *M* ammonium formate: glacial acetic acid (40:60:0.5) at a flow rate of 1.0 ml/min. Detection of the raclopride peak was by ultraviolet absorption at 254 nm. Raclopride eluted at approximately 9 min. A 50–100 μl standard of each supernatant was removed prior to HPLC injection and used to determine column recovery.

Image Analyses

For the purpose of drawing ROIs, we obtained an averaged emission scan representing the activity from 10–60 min post-injection of the tracer. ROIs for basal ganglia (BG) and cerebellum (CBL) were drawn on these averaged images and then projected to the dynamic emission scans. For the basal ganglia, a region for dorsal striatum was obtained by averaging activity in left and right basal ganglia in two sequential planes. To minimize inaccurate quantitation introduced by small differences in relative position of the basal ganglia within the z-axis of the gantry (*14*), we selected the two central sequential planes where the basal ganglia were located. Because the dimensions of the BG in the z-axis (2.5 cm) (*15*) are smaller than twice the FWHM in this axis (1.3 cm), use of the central slices minimizes errors introduced by varying their recovery coefficient from one study to the other as a result of misposition. To further minimize errors from partial volume effects in the other planes, we obtained ROIs with volumes smaller than twice the FWHM in the axial and transverse planes (BG = 0.99 cm^3 and CBL = 1.32 cm^3). For the CBL, we averaged the values obtained in left and right cerebellar regions selected from the slice located in the middle of the cerebellum. Figure 1 shows the shape, size and location of the regions selected.

Reproducibility of ^{11}C-raclopride binding was assessed for the following quantitative measurements:

1. Time-activity curves for ^{11}C-raclopride in BG and CBL were quantitated to obtain peak uptake expressed as %dose/cc, time to reach peak uptake and percent clearance from peak uptake at 60 min.
2. BG-to-CBL ratios were obtained by averaging the activity in these regions comprising the 30- to 60-min period after injection of tracer.
3. The distribution volume (DV) was obtained using a graphical analyses technique for reversible system as previously described (*16*). The ratio of the DV in BG to that in CBL was used to assess reproducibility of the measurements.

The DV provides a measure of binding that is a linear function of receptor availability given by

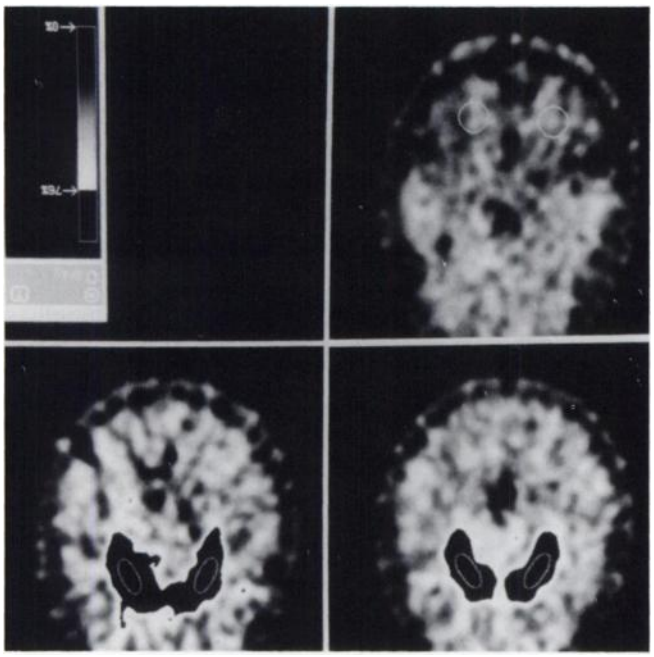

FIGURE 1. Location, size and shape of ROIs used to quantitate ^{11}C-raclopride in basal ganglia (upper images) and cerebellum (lower image).

$$DV = K_1/k_2'(1 + NS + Bmax/Kd), \quad \text{Eq. 1}$$

for regions containing receptors characterized by an equilibrium dissociation constant Kd and free receptor concentration, Bmax. For nonreceptor regions the DV is given by

$$DV = K_1/k_2'(1 + NS). \quad \text{Eq. 2}$$

In both equations, NS represents the ratio of transfer constants for nonspecific binding, K_1 and k_2' are the plasma to tissue and the tissue to plasma transport constant respectively. A parameter proportional to Bmax can be obtained from Equations 1 and 2 giving

$$Bmax/K_d(1/1 + NS) = [DV(BG)/DV(CB)] - 1. \quad \text{Eq. 3}$$

Equations 1 and 2 are based on classical compartmental analysis in which the effects of CBF and capillary permeability are implicitly included in the parameters K_1 and k_2'. The advantage of the DV is that it is easily determined by a graphical technique derived from classical compartmental equations; it is not a function of blood flow (*17*) and is a more stable measure than the individual kinetic constants determined directly by compartmental analysis which are sensitive to noise and statistical fluctuations in the data (*18*). The ratio of DV for BG to CBL eliminates possible differences in the K_1/k_2' ratio between experiments. The assumption that the ratio of the transport constants is the same for BG and CBL is common (*19*).

Reproducibility in the values for BG/CBL and for the ratio of the DV in BG to that in CBL was estimated using intraclass correlation analysis (*20*). This correlation with range 0 (no stability) to 1 (perfect stability) quantitates the ability of a measurement to characterize true differences between individuals relative to measurement variability. It is interpreted as an index of measurement reliability in the statistical literature (*20,21*).

RESULTS

The individual values for the mass of raclopride injected and for the integrated plasma concentration for total radioactivity and for unchanged ^{11}C-raclopride for the two studies are shown in Table 1. For all of the studies, the mass of ^{11}C-raclopride injected was within a tracer dose range. Analysis of plasma revealed no significant differences in total plasma activity nor in percent nonmetabolized ^{11}C-raclopride between repeated studies. The percentage of unchanged ^{11}C-raclopride corresponded to 95% ± 4% at 1 min, 88% ± 3% at 10 min, 81% ± 4% at 30 min and 68% ± 3% at 60 min.

Time-activity curves for ^{11}C-raclopride in BG and CBL are shown in Figure 2. Peak activity was reached between 10–25 min, at which point radioactivity plateaued or gradually decreased. Peak uptake in BG varied between subjects from 0.0041% dose/cc to 0.0060% dose/cc. Peak uptake between repeated runs on a given subject ranged from 0% to 7% (Table 2). Percent clearance from time of peak to the end of study (60 min) showed a large intersubject variability, ranging from 13% to 43%. Within a given subject, the difference in percent clearance between repeated runs ranged from −9% to +4%.

The individual BG-to-CBL ratios for the repeated measures in the subjects are shown in Table 3. The average group values for the BG-to-CBL ratios for study 1 were 4.58 ± 0.46 and for study 2, 4.57 ± 0.55. Average percent change was 0.4% ± 5.4% and the intraclass correlation corresponded to r = 0.90.

The results from the graphical analyses are shown in Table 3. Average values for DV (expressed as milliliters

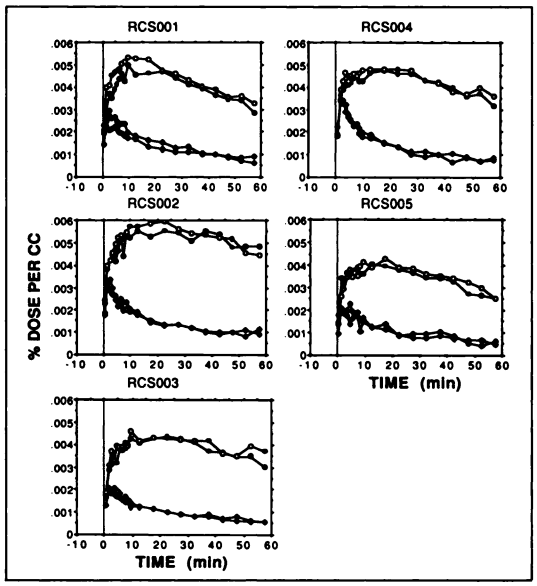

FIGURE 2. Individual time-activity curves for ^{11}C-raclopride in basal ganglia and cerebellum for studies 1 and 2. Activity is expressed as %dose/cc of tissue. For study 1: striatum, •; cerebellum, ◇. For study 2: striatum, ○; cerebellum, ◇.

TABLE 2
Analyses of Time-Activity Curves of ^{11}C-raclopride in Basal Ganglia for Studies 1 and 2

Subject	Study	Peak uptake %ID/cc	% Change	Time to peak uptake (min)	% Clearance at 60 min
rcs001	1	0.0050	+6	9.5	43
	2	0.0053		9.5	39
rcs002	1	0.0056	+7	22.5	13
	2	0.0060		22.5	16
rcs003	1	0.0043	+7	9.5	28
	2	0.0046		9.5	20
rcs004	1	0.0048	0	17.5	34
	2	0.0048		17.5	25
rcs005	1	0.0041	0	12.5	38
	2	0.0041		9.5	39

per gram of tissue) in BG and CBL were not significantly different between the two studies and corresponded in BG: study 1: 1.84 ± 0.03; study 2: 1.83 ± 0.2 and in CBL: study 1: 0.45 ± 0.1; study 2: 0.46 ± 0.1. The average group value for the ratio of the DV in BG to that in CBL was for study 1: 4.14 ± 0.5 and for study 2: 4.06 ± 0.5. Average percent change was −1.6% ± 6% and the intraclass correlation corresponded to r = 0.85.

DISCUSSION

This study shows that measurements of ^{11}C-raclopride in the human brain under conditions of no intervention are highly reproducible in the same individual on different days. The stability of ^{11}C-raclopride measurements as assessed with the BG-to-CBL ratio, and the ratio of the distribution volume in BG to that in CBL show individual variations of less than 10%. Although individual values for the distribution volume in BG were reproducible for four of the subjects, subject RCS002 showed a 16% variation. The variation for this subject was, in part, a function of an increase in the plasma concentration of unchanged ^{11}C-raclopride which also led to a 6% change in the distribution volume in CBL. In this respect, the use of the ratio of the distribution volume in BG to that in CBL compensates for global changes in ligand uptake. Even though one individual showed a variation of 9% for the model parameter representing B_{max}/K_d (DV BG/DV CBL), the test-retest changes were in both directions, with an average test-retest group variability of −1.6%. Because in an actual test situation (where an intervention is applied to change dopamine concentration) the changes will presumably occur in the same direction, the average test-retest group variability can be used to compare the group effects of the intervention.

Animal studies have demonstrated the sensitivity of ^{11}C-raclopride to pharmacological interventions that change intrasynaptic dopamine concentration (*6–10*). However, the stability of ^{11}C-raclopride binding when no interventions are made suggests that it is not sensitive to normal day-to-day fluctuations of the intrasynaptic concentration of dopamine. Its stability in humans makes it a potentially useful tracer for the investigation of changes in intrasynaptic dopamine brain concentration secondary to pharmacological and/or behavioral interventions. However, its sensitivity both to decreases as well as increases in dopamine concentration and dose response needs to be investigated.

In contrast to the stability of the measures in a given individual, there was considerable intersubject variability. Of particular interest is the variability in the rate of ^{11}C-raclopride clearance throughout the scanning period. The mechanisms that account for differences in rate of ^{11}C-raclopride clearance among individuals should be investigated to determine if this reflects differences in rate of dopamine release, blood flow, rate of free versus nonspecific and specifically bound ligand, differences in metabolic pattern of the ligand and/or differences in plasma delivery of ligand. We have observed a similar degree of intersubject variability in the rate of clearance of ^{11}C-raclopride in the baboon brain.

TABLE 3
Basal Ganglia-to-Cerebellar Ratios for ^{11}C-raclopride Uptake and Distribution Volumes for Studies 1 and 2

Subject	Study	Ratio BG/CBL	% Change	Distribution Volume			
				BG	CBL	DV BG/CBL	% Change
rcs001	1	4.06	−8	1.94	0.53	3.66	−7.02
	2	3.73		1.94	0.57	3.40	
rcs002	1	4.89	−2	2.21	0.47	4.70	−6.23
	2	4.80		1.94	0.44	4.41	
rcs003	1	5.21	−1	1.57	0.33	4.76	+2.71
	2	5.18		1.62	0.35	4.63	
rcs004	1	4.28	+2	1.99	0.55	3.62	+9.02
	2	4.36		2.13	0.54	3.94	
rcs005	1	4.47	+7	1.47	0.37	3.97	−1.26
	2	4.77		1.53	0.39	3.92	
Mean	1	4.58 ± 0.46	0.4 ± 5.4			4.14 ± 0.5	−1.64 ± 6
	2	4.57 ± 0.55				4.06 ± 0.5	

In considering these results, one has to realize that the reproducibility of ^{11}C-raclopride measurements was demonstrated when subjects were tested 24 hr apart at the same time of day and under the same experimental conditions. Repeated measurements at longer time intervals or at different times during the day may yield different results. Also, the stability of these measurements in patients with psychiatric and/or neurological disorders may vary as a function of the disease state and needs to be investigated.

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REFERENCES

1. Farde L, Ehrin E, Eriksson L, et al. Substituted benzamides as ligands for visualization of dopamine receptor binding in the human brain by positron emission tomography. *Proc Natl Acad Sci* 1985;82:8863–8867.
2. Farde L, Wiesel FA, Stone-Elander S, et al. D2 dopamine receptors in neuroleptic-naive schizophrenic patients; a positron emission tomography study with ^{11}C-raclopride. *Arch Gen Psychiatry* 1990;47:213–219.
3. Brooks DJ, Ibanez V, Sawle GV, et al. Striatal D2 receptor status in patients with Parkinson's disease, striatonigral degeneration and progressive supranuclear palsy measured with ^{11}C-raclopride and positron emission tomography. *Ann Neurol* 1992;31:184–192.
4. Seeman P, Grigoriadis DE, Niznik HB. Selectivity of agonists and antagonists at D2 dopamine receptors compared to D_1 and S_2 receptors. *Drug Dev Res* 1986;9:63–69.
5. Logan J, Dewey SL, Wolf AP, et al. Effects of endogenous dopamine on measures of ^{18}F N-methylspiroperiodol binding in the basal ganglia: comparison of simulations and experimental results from PET studies in baboons. *Synapse* 1991;9:195–207.
6. Seeman P, Guan HC, Niznik HB. Endogenous dopamine lowers the dopamine D2 receptor density as measured by ^{3}H raclopride: implications for positron emission tomography of the human brain. *Synapse* 1989;3: 96–97.
7. Young TL, Wong DF, Goldman S, et al. Effects of endogenous dopamine on kinetics of ^{3}H-N-methylspiperone and ^{3}H-raclopride binding in the rat brain. *Synapse* 1991;7:188–194.
8. Ross SB, Jackson DM. Kinetic properties of the accumulation of ^{3}H raclopride in the mouse in vivo Naunyn-Schmied. *Arch Pharmacol* 1989; 340:6–12.
9. Inoue O, Kobayashi K, Tsukada H, Itoh T, Langstrom B. Difference in in vivo receptor binding between ^{3}H-N-methylspiperone and ^{3}H-raclopride in reserpine-treated mouse brain. *J Neural Transm* 1991;85:1–10.
10. Dewey SL, Smith G, Logan J, et al. GABAergic inhibition of endogenous, dopamine release measured in vivo with ^{11}C-raclopride and positron emission tomography. *J Neuroscience* 1992;12:3773–3780.
11. Innis RB, Malison RT, Al-Tikrity M, et al. Amphetamine-stimulated dopamine release competes in vivo for ^{123}I-IBZM binding to the D2 receptor in nonhuman primates. *Synapse* 1992;10:177–184.
12. Kearfott KJ, Rottenberg DA, Knowles RJR. A new headholder for PET, CT and NMR imaging. *J Comput Assist Tomogr* 1984;8:1217–1220.
13. Farde L, Hall H, Ehrin E, Sedvall G. Quantitative analyses of D2 dopamine receptor binding in the living human brain by positron emission tomography. *Science* 1986;231:258–260.
14. Bendriem B, Dewey SL, Schlyer DJ, Wolf AP, Volkow ND. Quantitation of the human basal ganglia with positron emission tomography: a phantom study of the effect of contrast and axial positioning. *IEEE Trans Med Imag* 1991;10:216–222.
15. Blinkov SM, Glezer II. *The human brain in figures and tables. A quantitative handbook.* New York: Plenum. 1968:166–170.
16. Logan J, Fowler JS, Volkow ND, et al. Graphical analyses of reversible radioligand binding from time activity measurements applied to N-^{11}C-methyl-(-) cocaine PET studies in human subjects. *J Cereb Blood Flow Metab* 1989;10:740–747.
17. Holthoff VA, Koeppe RA, Frei KA, Paradise AH, Kuhl DE. Differentiation of radioligand delivery and binding in the brain:validation of a two compartment model for ^{11}C-flumazenil. *J Cereb Blood Flow Metab* 1991; 11:745–752.
18. Sawada Y, Hiraga S, Francis B, et al. Kinetic analysis of 3-Quinuclidinyl 4-[^{125}I]Iodobenzilate transport and specific binding to muscarinic receptor in rat brain in vivo: implications for human studies. *J Cereb Blood Flow Metab* 1990;10:781–807.
19. Wong DF, Gjedde A, Wagner HN. Quantification of neuroreceptors in the living human brain. I. Irreversible binding of ligands. *J Cereb Blood Flow Metab* 1986;6:136–146.
20. Haggard EA. *Intraclass correlation and the analysis of variance.* New York: Dryden Press; 1958.
21. Winer BJ. *Statistical principles in experimental design.* New York: McGraw-Hill; 1972.