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RESEARCH ARTICLE

Imaging Dopamine Receptors in the Human Brain by Positron Tomography

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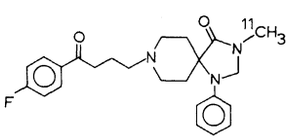
Abstract. *Neurotransmitter receptors may be involved in a number of neuropsychiatric disease states. The ligand 3-*N-[^{11}C]*methylspiperone, which preferentially binds to dopamine receptors in vivo, was used to image the receptors by positron emission tomography scanning in baboons and in humans. This technique holds promise for noninvasive clinical studies of dopamine receptors in humans.*

One of the most intriguing problems in biomedical research today is that of relating manifestations of neuropsychiatric diseases to chemical processes in different parts of the brain. The neurotransmitter dopamine appears to be associated with abnormalities related to disorders such as Parkinson's disease and schizophrenia. The highest density of dopamine neurons occurs in the nigrostriatal dopamine pathway which degenerates in Parkinson's disease (*1*). Neuroleptic drugs elicit extrapyramidal parkinsonian side effects by blocking dopamine receptors in the corpus striatum and also exert antischizophrenic action by blocking dopamine receptors, perhaps in limbic areas (*2*). Numbers of dopamine receptors are increased by chronic neuroleptic treatment (*3*) and are also increased in some schizophrenics, perhaps as a result of neuroleptic therapy (*4*).

The development of positron emission tomography (PET) and appropriate radioactive tracers labeled with positron-emitting radionuclides has now made it possible to relate regional biochemistry within the human brain to measurements of behavior in normal subjects and to elucidate abnormalities in patients with Alzheimer's disease (*5*), Huntington's disease (*6*), depression (*7*), and multiple infarct dementia (*8*). The technique consists of intravenous injection of a substance such as ^{18}F-labeled deoxyglucose, [^{11}C]carboxyhemoglobin, ionic rubidium-82, ^{68}Ga-labeled EDTA, and other radiopharmaceuticals, and subsequent imaging of the distribution of the radioactive label in the brain by means of the tomographic method, based on detection of the annihilation radiation produced during positron emission (*9*).

The butyrophenone neuroleptic drug spiperone has been useful in binding studies for measuring dopamine receptors both in vitro (*10*) and in vivo (*11*). We now report initial results obtained with 3-*N*-[^{11}C]methylspiperone (^{11}C-NMSP), a spiperone derivative, in PET



scanning studies to visualize the distribution of dopamine receptors in the brains of baboons and a human being. All studies were performed with a NeuroECAT scanner (Ortec, Inc., Oak Ridge, Tennessee), which has a spatial resolution of approximately 8 mm (full width at half maximum) in the plane of the slice. The distance between slices is 3 cm.

The newly developed tracer ^{11}C-NMSP was synthesized by *N*-alkylation of spiperone with [^{11}C]methyl iodide; the iodide was produced from $^{11}CO_2$, which in turn had been produced with an in-hospital cyclotron (model RNP-16, Scanditronix Cyclotron, Sweden). Carbon-11 is a positron-emitting isotope with a physical half-life of 20 minutes. The entire synthesis was accomplished with material ready for injection within 55 minutes after the end of the cyclotron

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bombardment. Prior to injection, the product was purified and the specific activity was determined by using a reverse-phase liquid chromatographic column (EM brand Lichrosorb column RP 18). The specific activity was also determined by an in vitro competitive binding assay. Although the nonradioactive and radioactive syntheses were carried out by new methods (*12*), the compound was previously reported in a patent by Janssen (*13*).

Whether a ligand is binding to receptors in vivo can be determined by testing whether the regional distribution of radioactivity after drug injection parallels the distribution of receptors, and also by testing whether administration of excess, unlabeled, related drugs block this specific regional distribution. For example, the rat striatum has very high concentrations of dopamine receptors while the cerebellum has very low levels (*11*). Thus, high striatal/cerebellar ratios of injected drug indicate the successful, preferential labeling of dopamine receptors in vivo (*11*). The content of drug in the cerebellum is a measure of nonspecific or non-receptor-associated drug in brain while the striatal content reflects total drug, that is, both specific and nonspecific binding. Administration of excess unlabeled neuroleptic drugs with high affinity for dopamine receptors obliterates the regional distribution (*11*).

In experiments in vitro, we found that NMSP has a binding affinity for dopamine receptors similar to that of spiperone (*14*). In preliminary studies in vivo, we tested whether NMSP would be localized at dopamine receptors as spiperone is. [^{11}C]NMSP (55 mCi/μmole; the dose was 10 μg per kilogram of body weight) was injected into the tail veins of mice. After sacrificing the animals and dissecting the brain regions, we found that striatal activity was 15 to 20 times greater than cerebellar radioactivity at 60 minutes after injection. Coinjection of unlabeled spiperone lowered the high striatal/cerebellar ratios by 70 percent at spiperone doses of 150 μg/kg without altering cerebellar binding. These biochemical studies indicate that NMSP is a suitable ligand for labeling dopamine receptors in vivo.

The next experiments involved PET imaging in anesthetized baboons. Intravenous injections of 16 mCi of [^{11}C]NMSP (specific activity, 223 Ci/mmole) were administered in three separate studies. Preferential accumulation of radioactivity in the caudate nucleus and putamen was observed as early as 15 minutes after injection in scans on all occasions.

In one of these studies, two injections separated by 3 hours were given. The first injection consisted of [^{11}C]NMSP with a specific activity of 10.4 mCi/μmole (the dose was 21 μg/kg). The injected tracer selectively concentrated in the region of the caudate nucleus and putamen relative to the rest of the brain. After the carbon-11 had been allowed to

Table 1. 3-*N*-[^{11}C]Methylspiperone levels in baboon caudate nucleus and cerebellum. Regions for quantitation were selected from the video monitor with a cursor and verified by comparison to CT scans. Scan 1 was made 40 to 60 minutes after the first injection; scan 2 was 40 to 60 minutes after the second injection. All counts are corrected back to time of injection. Data are from one of three studies.

Scan	Condition	Mean counts per minute per pixel*		Caudate/cerebellum ratio
		Caudate	Cerebellum	
1	[^{11}C]NMSP alone	303	149	2.03
2	[^{11}C]NMSP with excess unlabeled spiperone	93	88	1.05

*Mean counts per minute per picture element of computer.

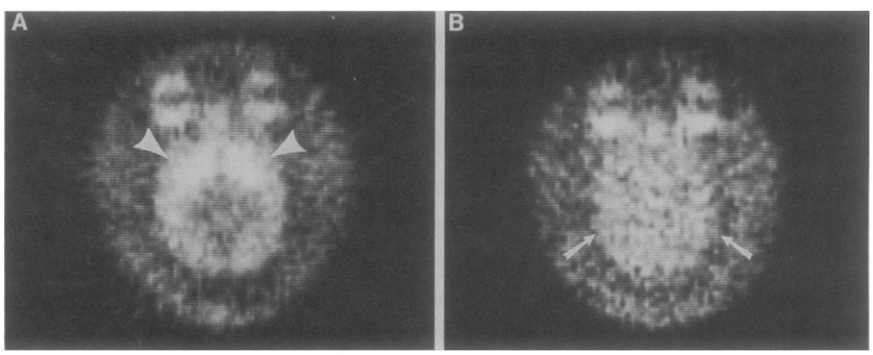

Fig. 1. PET images of baboon brain following intravenous injection of [^{11}C]NMSP. A 9-year-old, 30-kg male *Papio anubis*, obtained from Primate Imports, was initially immobilized with ketamine (250 mg, intramuscular), anesthetized with sodium pentobarbital (15 mg/kg, intravenous), and received atropine (0.2 mg, intramuscular). The animal was positioned by using anatomic and surface markings such that the middle slide of the NeuroECAT passed through the caudate heads and cerebellum. High-resolution (full width at half-maximum = 9 mm) acquisitions were made with septa and shadow shields in and a Shepp and Logan filter. Calculated attenuation corrections were made by using an elliptical region of interest. (A) PET scan obtained 40 to 60 minutes after injection, showing relatively increased activity in the basal ganglia following the injection of [^{11}C]NMSP (16 mCi; 10.4 mCi/μmole) in the baboon (arrowheads). (B) The same PET section 40 to 60 minutes after injection of an excess of unlabeled spiperone (6.6 mg; 222 μg/kg), along with [^{11}C]NMSP. Compared to (A) this image shows uniform uptake throughout the brain (arrows). This indicates blockade of the dopamine receptors by unlabeled drug, resulting in little or no specific receptor binding in the area of the caudate nucleus. Both displayed images were scaled by the computer to a fixed maximum brightness, giving an artificial appearance of higher counts in the whole brain in (B). The actual counts were considerably lower than those in (A); see Table 1.

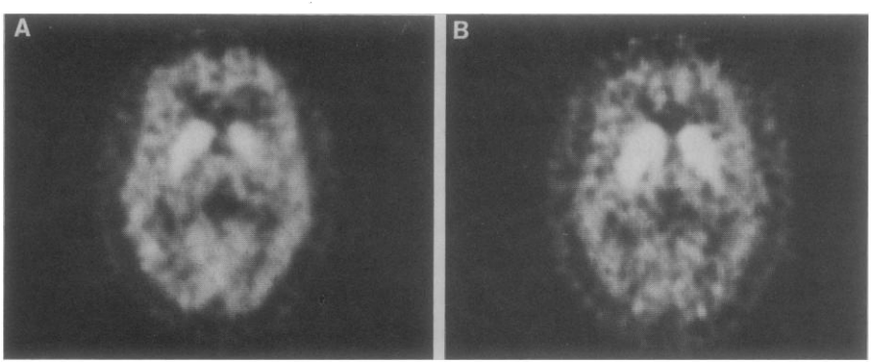

Fig. 2. PET images of a human brain after intravenous injection of [^{11}C]NMSP. An awake, 56-year-old Caucasian male was positioned in the PET scanner by use of a headholder. The middle slice of the PET scan contained a section of the brain including the basal ganglia. The scan was carried out in the same way as with the baboons. Images obtained 40 to 60 minutes after injection (A) and 70 to 130 minutes after injection (B) show a high accumulation of activity in the basal ganglia relative to the rest of the brain.

decay to insignificant levels for 3 hours (nine half-lives) and while the baboon was still anesthetized, a second dose of [^{11}C]NMSP was injected intravenously, but this time with an added excess (220 μg/kg) of unlabeled spiperone which would compete for binding to the receptors. The activity in this instance did not accumulate preferentially in the region of the caudate nucleus and putamen, although it again accumulated in the eyes as it had previously (Table 1 and Fig. 1). The reduction in binding in caudate and putamen elicited by unlabeled spiperone treatment indicates pharmacological specificity of the dopamine receptor binding in this area, while the lack of reduction in the eye region indicates a very large fraction of nonspecific binding in the latter area.

After the baboon experiments, the following experiment was performed with one of us (H.N.W.) as the experimental subject. Twenty millicuries of [^{11}C]-NMSP was injected intravenously in the conscious subject while he lay supine with his eyes closed and his head in the PET scanner. The specific activity of the [^{11}C]NMSP was 263 mCi/μmole and the injected dose was 70 μg (approximately 1 μg/kg). There was a progressive increase with time in the caudate/cerebellar ratio of activity as visualized by serial PET scans (Table 2 and Fig. 2). This has been observed in many animal studies and is due mainly to the reduction of nonspecific binding in the cerebellum and remainder of brain (*11*). Figure 2, A and B, shows the unprocessed PET images. The basal ganglia were again seen, with slightly less activity in the rest of the brain. Activity in other regions, particularly in the cerebral cortex, could reflect serotonin-2 receptors, which are also labeled by neuroleptic drugs (*15*).

The planes for PET imaging in the human and baboon studies were selected after x-ray computed tomography (CT) images identified planes for examining the basal ganglia. Moreover, the location of the high concentrations of activity within the brain slice corresponded to the location of the basal ganglia as determined by the CT scans. A headholder was used to ensure ease and reproducibility of locating the planes. The eyes were out of the planes imaged in the human study (*16*). The caudate/cerebellar ratio of radioactivity was 4 at 70 to 130 minutes after injection in the human and 2 at 40 to 60 minutes in baboons. These ratios are underestimates of the true activity distribution because of physical factors including partial volume and resolution effects (*17*).

In summary, [^{11}C]NMSP, a compound with a high affinity for dopamine receptors in vitro, is localized in the basal ganglia, a region with a high density of dopamine receptors, after in vivo administration in primates. The enrichment in the basal ganglia is blocked by administering other nonradiolabeled dopamine receptor–blocking drugs in excess. Taken together, these data indicate that the imaged radioactivity reflects dopamine receptor densities. Although other laboratories have used drugs labeled with positron-emitting isotopes with the goal of identifying dopamine receptors in vivo (*18*), to our knowledge, this study, performed on 25 May 1983, was the first demonstration that it is possible to image the distribution of dopamine receptors in human brain. It may now be feasible to assess in humans the role of dopamine receptors in the actions of numerous psychotropic drugs and in disorders such as schizophrenia, Parkinson's disease, tardive dyskinesia, and Huntington's disease.

Table 2. 3-*N*-[^{14}C]Methylspiperone levels in human caudate nucleus and cerebellum. All counts are corrected back to time of injection.

Scan	Time of acquisition after injection (min)	Mean counts per minute per pixel		Caudate/cerebellum ratio
		Caudate	Cerebellum	
1	20 to 30	110	63	1.8
2	40 to 60	117	42	2.8
3	70 to 130	86	20	4.4

Note added in proof: Since the submission of this manuscript, we have carried out similar studies in four additional normal persons with essentially the same results.

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12. The synthetic chemistry and radiochemistry will be described in detail in future publications.
13. P. A. Janssen, patent 3155670 (1964).
14. In binding experiments in vitro we found that NMSP has a K_i of about 250 p*M* (against [^{3}H]spiperone) while the K_D of spiperone under the same conditions was about 190 p*M*. More extensive binding studies of radiolabeled NMSP in vitro will be published elsewhere.
15. Since spiperone has a significant affinity for serotonin-2 receptors [S. J. Peroutka and S. H. Snyder, *Mol. Pharmacol.* **16**, 687 (1979)], we tested whether NMSP had any affinity for these receptors. In binding experiments with rat frontal cortex in vitro, we found that NMSP had a K_i for serotonin-2 receptors (against [^{3}H]spiperone) of about 1.3 n*M*, while spiperone had a K_D for these receptors of about 0.8 n*M* under the same conditions. Thus, NMSP has a high affinity for serotonin-2 receptors, although it is somewhat less than that of spiperone. While some of the radioactivity imaged in the PET scans is presumably localized at serotonin-2 receptors, the bulk of the activity is localized at dopamine receptors. This is clear from the fact that the activity concentrates highly in the caudate and putamen compared to the cortex; this parallels the distribution of dopamine receptors rather than serotonin-2 receptors, which are found in about equal concentrations in the caudate and cortex in several species [S. J. Peroutka and S. H. Snyder, *Brain Res.* **208**, 339 (1981)]. Also, the affinity of NMSP for dopamine receptors is about five times that for serotonin-2 receptors [see above (*14*)].
16. Three simultaneous planes (slices 32 mm apart) were acquired for the PET scans. For the human studies the cerebellum was on a plane 32 mm caudal to the plane with the caudate (Fig. 3); it is not shown here but was used for the count determinations in the region of interest.
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