In Vivo Labeling of the Dopamine D2 Receptor with N-^{11}C-Methyl-Benperidol

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A new dopamine D2 receptor radiotracer, N-^{11}C-methyl-benperidol (^{11}C-NMB), was prepared and its in vivo biologic behavior in mice and a baboon was studied. Carbon-11-NMB was determined to bind to specific sites characterized as dopamine D2 receptors. The binding was saturable, reversible, and stereospecific. Kinetic studies in the dopamine D2 receptor-rich striatum showed that ^{11}C-NMB was retained five times longer than in receptor-devoid regions, resulting in a high maximum striatal-to-cerebellar ratio of 11:1 at 60 min after injection. From frontal cortex and cortex, on the other hand, the tracer washed out as rapidly as it did from cerebellum, resulting in tissue-to-cerebellar ratios close to one in these regions at any time after injection. Blocking studies confirmed the specificity and selectivity of the ^{11}C-NMB binding to the dopamine D2 receptor. A PET study with ^{11}C-NMB of the baboon brain revealed highly selective labeling of dopamine D2 receptor sites which was blocked by preinjection of raclopride.

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The dopamine D2 receptor in the living brain has been visualized by the positron emission tomographic (PET) technique using carbon-11- (^{11}C), fluorine-18-, and bromine-76- (^{76}Br) labeled neuroleptic drugs as radiotracers. Among these drugs, spiperone, a butyrophenone dopamine antagonist of high affinity for the receptor with a Kd value of 0.14 n*M* (*1*), its alkylated (*2–5*) and brominated (*6,7*) derivatives have been evaluated as in vivo ligands and have found widespread use for studying the dopamine D2 receptor (*8–10*). With these compounds, the receptor-rich regions in the brain can be clearly visualized with PET. However, it is well recognized that spiperone and its derivatives have multiple specific binding sites other than the dopamine D2 receptor (*1,11–15*). In particular, the binding of spiperone to the serotonin S2 receptor in frontal cortex has been reported to be close to 50% of the amount bound to the dopamine receptor in striatum (*11*). With the methylated derivative, N-methyl-spiperone, ~20% of the binding of this compound in the striatum and 90% in the frontal cortex was characterized as binding to the serotonin S2 receptor (*15*). As a result, in PET brain images with spiperone or its derivatives, the tissues with low densities of dopamine receptors but an abundance of serotonin S2 receptors, such as frontal cortex and cortex, are visualized as well as dopamine receptor-rich tissues.

This poor selectivity is a disadvantage of these ligands as dopamine D2 receptor radiotracers. Among the series of the butyrophenone neuroleptic compounds developed by Janssen et al. (*16*), haloperidol and benperidol have better selectivity (*12,16,17*) than spiperone. Haloperidol, however, revealed high uptake in tissues (e.g., the cerebellum), which are devoid of dopamine D2 receptors. As a result, ^{3}H or ^{18}F-haloperidol labels the brain almost uniformly in vivo (*1,18*). Benperidol has high affinity for the dopamine D2 receptor (*1*) and was classified by Janssen et al. as a neuroleptic drug of high apomorphine antagonistic effect and low anti-tryptamine and anti-norepinephrine potencies (*16*). Fluorine-18-benperidol has been synthesized and evaluated in the baboon brain and has shown high target-to-non-target ratios (*19*). A brominated derivative ^{77}Br-brombenperidol has also been reported (*20*). However, this compound exhibited relatively low in vivo target-to-non-target ratios.

This report describes the in vivo kinetics, distribution, and pharmacology of N-^{11}C-methyl-benperidol (^{11}C-NMB), a derivative of benperidol, as a potentially more selective radiotracer of the dopamine D2 receptor.

MATERIALS AND METHODS

Benperidol was prepared according to literature procedures (*21*). N-methyl-benperidol (NMB) was synthesized by a procedure similar to the one described below. (+)-Butaclamol, (−)-butaclamol, ketanserin, SCH-23390, spiperone, and haloperidol were purchased from Research Biochemicals Inc., Natick, MA. Raclopride was gratefully obtained as a gift from Astra Alab AB, Södertälje, Sweden.

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Radiotracer Synthesis

Carbon-11-NMB was synthesized by N-methylation of the precursor benperidol with ^{11}C-iodomethane (*22*). Briefly, benperidol and ^{11}C-iodomethane were reacted at 80°C for 1 min in dimethylformamide. Carbon-111-NMB was purified by reversed-phase high-performance liquid chromatography (HPLC). After evaporation of the HPLC solvent, the labeled compound was dissolved in normal saline and sodium bicarbonate and filtered for injection. Radiochemical yields were 20%–50% with a radiochemical purity of 100%. The specific activities were determined to be ~2200 mCi/μmole at the end of synthesis. The full synthetic details will be reported elsewhere (unpublished results). N-^{11}C-methyl-spiperone (^{11}C-NMSP) was synthesized as previously reported (*23*).

Brain Biodistribution Study

Mice weighing between 25 to 30 g were injected intravenously via tail vein with 110–160 μCi of ^{11}C-NMB (this corresponds to a dose of 1.6 ± 0.4 μg NMB/kg body weight). The mice were killed by cervical dislocation at 5, 15, 30, 60, and 90 min after injection and blood (100 μl) was collected from the carotid artery. The brains were removed, placed on ice, and dissected into the following five regions: cerebellum, striatum, frontal cortex, remaining cortex except the area superior to the corpus striatum, and the rest of the brain. After each tissue was weighed, the radioactivity concentration in these tissues and in the blood was determined using an automated gamma counter.

Blocking Study

The selectivity of ^{11}C-NMB binding to dopamine D2 receptor sites was examined as follows. Mice were injected intravenously with 1 mg/kg of (+)-butaclamol, (−)-butaclamol, ketanserin, SCH-23390, prazosin, propranolol, spiperone, raclopride (1–5 mg/kg), haloperidol, or unlabeled NMB. The drugs were dissolved in saline and administered at 15 min prior to the ^{11}C-NMB injection. The animals were once again killed by cervical dislocation 15 min after the injection of the radiotracer.

Comparative blocking experiments were performed with another dopamine D2 radiotracer, ^{11}C-NMSP. The mice in these experiments were killed at 30 min after injection of ^{11}C-NMSP. This time point (instead of 15 min for ^{11}C-NMB) was chosen because of the slower in vivo kinetics of ^{11}C-NMSP.

Statistical evaluation of the data was performed by the Student's t-test.

Baboon PET Study

A 30-kg male baboon (Papio Anubis) was anesthetized with alfadolone and alfaxolone acetate. Anesthesia was maintained throughout the study by continuous i.v. infusion drip of 6–9 mg/kg/hr (20% solution). Twenty millicuries of high-specific activity ^{11}C-NMB (2250 mCi/μmole) were administered intravenously. In a competition study, raclopride (2 mg/kg) was injected i.v. 5 min prior to the ^{11}C-NMB injection. Sequential tomographic slices passing through the caudates, cerebellum, and temporal lobes were obtained over a period of 110 min. Regions of interest (3 × 3 pixels) were placed over the different areas of the baboon brain based on a baboon PET atlas (*24*).

In Vitro Experiments

To evaluate the affinity of NMB for the dopamine D2 and serotonin S2 receptors, in vitro receptor assays were performed according to the following procedures. For both receptor assays, rats were killed by decapitation and the brains were removed quickly.

For the dopamine D2 receptor assay, striata were dissected and homogenized in ice-cold 50 m*M* Tris buffer (pH 7.4 at 37°C). The homogenate was centrifuged at 48,000 g for 15 min. The pellets were suspended in ice-cold buffer and rehomogenized. After recentrifugation, a tissue suspension of 6 mg/ml of buffer was made. To glass test tubes, 0.1 ml of 6 n*M* ^{3}H-spiperone (32.4 Ci/mmol; NEN, N. Billerica, MA) in buffer and 0.1 ml of a 10^{-4} to 10^{-9} *M* NMB solution were placed; then, 0.8 ml of the tissue suspension was added. For comparison, samples with 10^{-4} to 10^{-9} *M* benperidol were also prepared. For determination of nonspecific binding, 1 μ*M* (+)butaclamol was used. The samples were incubated at 37°C for 20 min. At the end of incubation, 3 ml of the ice-cold buffer were added. Bound and free radioligands were separated by pouring the samples onto GF/B glass filter discs (Whatman) under vacuum. The discs were washed three times with 3 ml of buffer and placed in glass vials, to which 10 ml scintillation cocktail (NEN NEF-963) was added. The radioactivity concentrations on the filter discs were determined using liquid scintillation counting.

To evaluate the affinity for the serotonin S2 receptor, receptor assays were performed in ice-cold 50 m*M* Tris buffer containing 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$, 10 m*M* $MgCl_2$, and 0.5 m*M* EDTA (pH 7.7) using rat frontal cortex as the tissue and ^{3}H-ketanserin (64.9 Ci/mmol) as the radioligand. Nonspecific binding was determined with 5 μ*M* cinanserin. Assay procedures were similar to the dopamine D2 receptor assay.

Data analysis to determine IC_{50} and K_i (inhibition constant) values was performed using microcomputer programs by McPherson (*25,26*).

RESULTS

After injection of ^{11}C-NMB into mice, the time course of ^{11}C radioactivity in cerebellum, striatum, frontal cortex, and remaining cortex was determined (Fig. 1). As early as 5 min after injection, a high uptake by the striatum was observed. The radioactivity reached a peak of 12% dose per gram of tissue at 15 min and declined exponentially with a slope of 0.01 min^{-1}, which corresponded to a washout rate of ~0.1% dose/g/min or 2–6 pg/g/min. The biologic half-life of ^{11}C-NMB in the striatum was 62 min. In contrast, the radioactivity in cerebellum, frontal cortex, and remaining cortex washed out rapidly. The biologic half-lives in these tissues were 12, 12, and 13 min, respectively, which were ~5 times shorter than in the striatum. As a result, at 30 min after injection, the radioactivity concentrations in these three tissues (cerebellum, frontal cortex, and remaining cortex) were as low as 1.4% injected dose per gram of tissue while there still remained 11% dose per gram in the striatum. The time course of the tissue to cerebellar ratios is shown in Figure 2. The ratios of frontal cortex-to-cerebellum and of remaining cortex-to-cerebellum were 1.15 ± 0.14 (n = 15) and

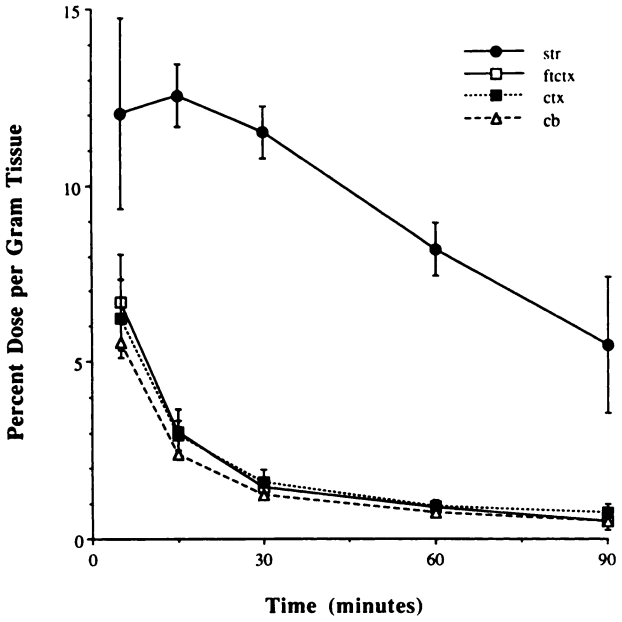

FIGURE 1
Time course of ^{11}C radioactivity in mouse striatum (str), frontal cortex (ftctx), cortex (ctx), and cerebellum (cb) after i.v. injection of ^{11}C-NMB (110–160 μCi, 1.6 ± 0.4 μg). Data are mean % dose/g ± s.d. (n = 3–5).

1.24 ± 0.11 (n = 15), respectively, at any time after injection (5–90 min). In contrast, striatal-to-cerebellar ratios increased with time until a plateau value of 11:1 was reached at ~60 min after injection.

In vivo characterization of the ^{11}C-NMB binding was carried out by studying the effect of several dopamine D2 receptor blockers as well as drugs with high affinity for dopamine D1, serotonin S2, and α-adrenergic sites (Fig. 3). Two dopamine D2 receptor ligands, spiperone and haloperidol, blocked the accumulation of ^{11}C-NMB in the striatum by 99% and 97%, respectively, indicating high specificity of ^{11}C-NMB binding to dopamine D2 receptor sites in striatum. (+)-Butaclamol blocked 100% of the specific binding while (−)-butaclamol did not ($p > 0.5$); this suggests that the binding of ^{11}C-NMB to the dopamine D2 binding site in striatum is stereospecific (*27*). Unlabeled NMB (1000 times the amount injected during the control studies) blocked 99% of the ^{11}C-NMB specific binding; this demonstrates the saturability of the NMB binding site in the striatum. Raclopride, a dopamine D2 receptor antagonist of a different type (*14,28,29*), inhibited 87.7% ± 3.9% of the ^{11}C-NMB binding at a dose of 1 mg/kg (2.9 μmole/kg).

SCH-23390, a dopamine D1 receptor-specific ligand (*30–32*), showed a consistent blockade of 20% ($p < 0.0001$). Ketanserin, a ligand for the serotonin S2 receptor (*17*), did not cause significant inhibition either in striatum ($p > 0.1$) (Fig. 3), in frontal cortex ($p > 0.1$), or the remaining cortex ($p > 0.1$) (data not shown). The α1 and α2 adrenergic blockers, prazosin and propran-

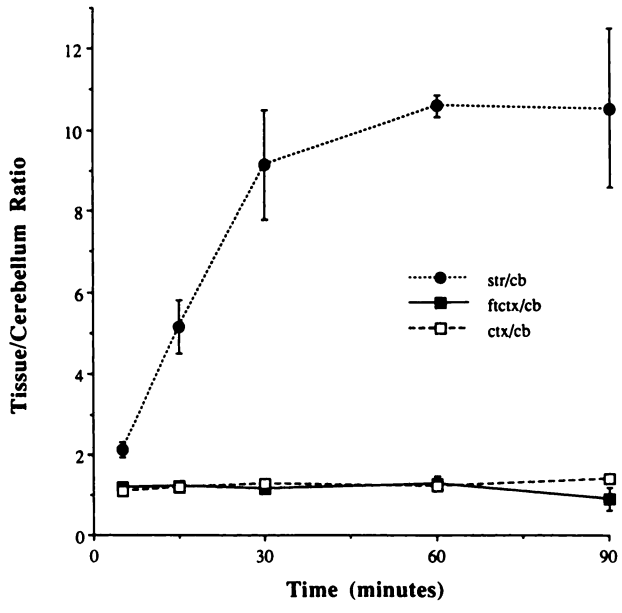

FIGURE 2
Time course of tissue to cerebellar ratios of ^{11}C radioactivity after i.v. injection of ^{11}C-NMB. Data are mean ratios ± s.d. (n = 3–5).

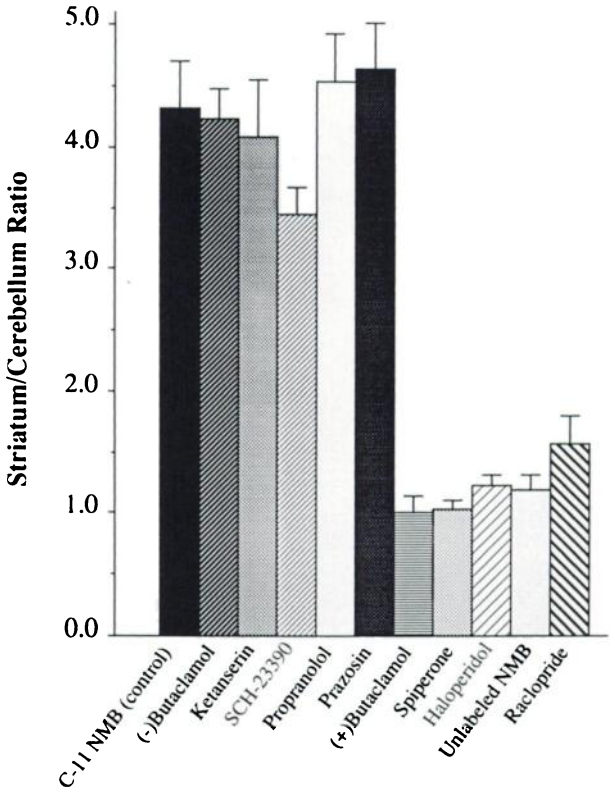

FIGURE 3
Effect of various drugs on ^{11}C-NMB binding in the mouse striatum. Drugs (1–5 mg/kg) were injected intravenously 15 min before administration of ^{11}C-NMB. Striatal to cerebellar ratios were determined 15 min after radiotracer application. Data are mean ratios ± s.d. (n = 4–12).

olol (*33*), respectively, also did not block the accumulation of ^{11}C-NMB ($p > 0.1$ for both drugs).

In the frontal cortex and the rest of the cortex, none of the drugs tested had any effect on ^{11}C-NMB binding ($p > 0.05$). Mean tissue-to-cerebellar ratios after blocking were 1.13 ± 0.10 ($n = 30$) for the frontal cortex and 1.10 ± 0.06 ($n = 30$) for the cortex.

Carbon-11-NMSP on the other hand showed quite different binding characteristics in striatum, frontal cortex, and cortex (Fig. 4) than ^{11}C-NMB. Whereas striatum-to-cerebellar ratios of ^{11}C-NMSP at 30 min after injection were comparable to those observed with ^{11}C-NMB, the ratios of frontal cortex-to-cerebellum and of cortex-to-cerebellum were six and three times higher (7.3 ± 0.7 and 3.9 ± 0.3), respectively. The specific binding of ^{11}C-NMSP in the striatum was not blocked significantly by ketanserin ($p > 0.5$) while 79% of the binding in the frontal cortex and 76% in the cortex were blocked by this drug, which suggests that NMSP binding in these tissues was mostly to serotonin S2 receptor sites. SCH-23390, the dopamine D1 receptor ligand which has been reported to interact with the serotonin S2 receptor (*31,32*), also showed 66% and 55% blockade in frontal cortex and cortex, respectively, while no significant blockade was seen in the striatum ($p > 0.1$). Raclopride at a dose of 1 mg/kg (2.9 μmole/kg) blocked only 55% of the ^{11}C-NMSP binding sites in the striatum. With a higher dose (10 mg/kg), 83% of these sites were blocked. However, at this dosage, significant blockade was observed in frontal cortex and the remaining cortex as well as in the striatum, while at a lower dose of 1–5 mg/kg, no significant blockade was observed in cortical areas. Unlabeled NMB (1 mg/kg or 2.5 μmole/kg) inhibited ^{11}C-NMSP binding in the striatum by 75%. In frontal cortex and remaining cortex, no significant effects were observed with this compound ($p > 0.1$).

After evaluation of ^{11}C-NMB in mice, the compound was further tested in a baboon. The corpus striatum was the area of highest accumulation of ^{11}C-NMB (Fig. 5). The activity increased gradually and striatal-to-cerebellar ratios of 2.0:1 were observed between 60 and 90 min after injection (Fig. 6a). There was no apparent accumulation of ^{11}C-NMB in the frontal cortex or other cortical regions, which suggests that this tracer retained its specificity of binding in this species (Fig. 6a). After pretreatment with 2 mg/kg of raclopride, the striatal uptake of ^{11}C-NMB was significantly inhibited (striatum-to-cerebellar ratio = 1:1; Fig. 6b).

In vitro receptor assays revealed that K_i values against ^{3}H-spiperone binding to rat striatum were 5.2 n*M* for NMB and 1.1 n*M* for benperidol. They also revealed that NMB had a significantly lower affinity for the serotonin S2 receptor (K_i = 50 n*M*) than benperidol (K_i = 4.0 n*M*) and NMSP (K_i = 0.45 n*M*).

DISCUSSION

This investigation demonstrates specific and selective in vivo labeling of the dopamine D2 receptor by ^{11}C-NMB. The binding was found to be saturable, reversible, and stereospecific. Because of its high selectivity, specificity and reversibility, ^{11}C-NMB may be a more appropriate dopamine D2 receptor radiotracer than ^{11}C-NMSP.

At all times after i.v. injection, ^{11}C-NMB concentrations were highest in the dopamine D2 receptor-rich striatal region (Fig. 1). Unlike ^{11}C-NMSP, ^{11}C-NMB

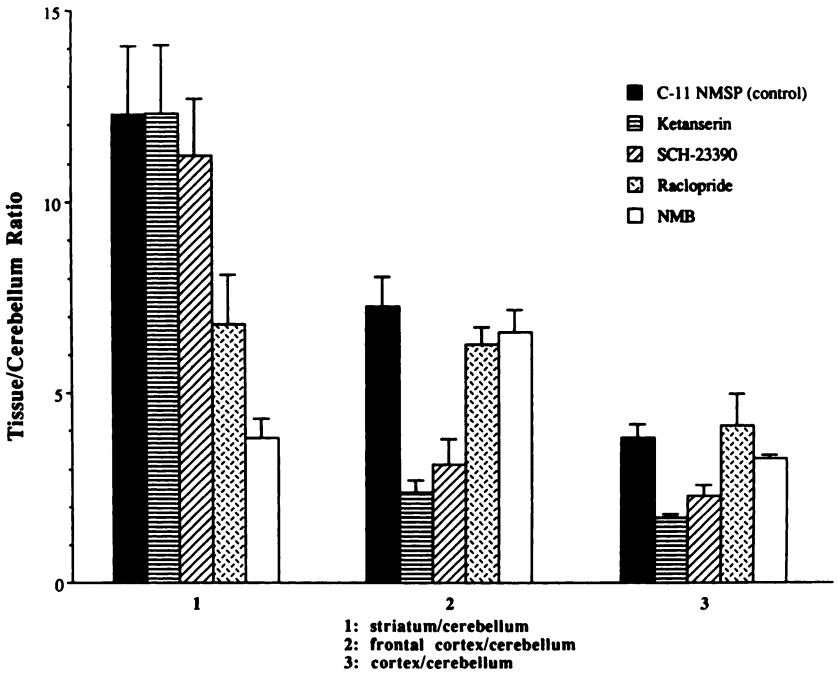

FIGURE 4
Effect of various drugs on ^{11}C-NMSP binding in different areas of the mouse brain. Drugs (1–5 mg/kg) were injected intravenously 15 min before administration of ^{11}C-NMSP. Tissue-to-cerebellar ratios were determined 30 min after radiotracer application. Data are mean ratios ± s.d. ($n = 3–4$).

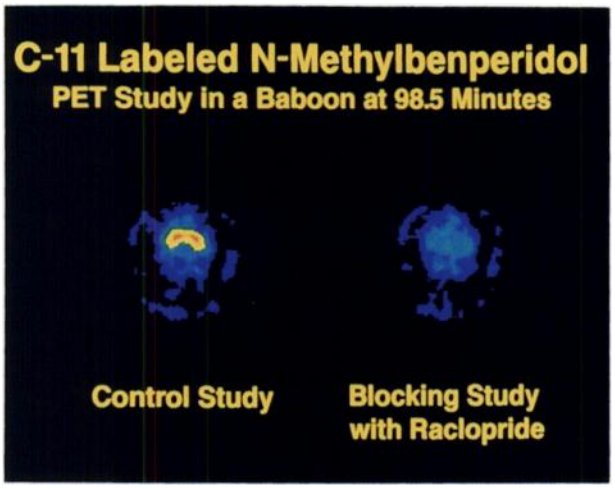

FIGURE 5
PET studies in baboon 98.5 min after injection of ^{11}C-NMB. Control study on right and blocking study by preinjection on raclopride on left.

showed no specific binding in the frontal cortex or remaining cortex, which are the tissues abundant in serotonin S2 or α-adrenergic receptors (Figs. 1 and 6a). From these brain regions, ^{11}C-NMB washed out as rapidly as from the cerebellum, a region devoid of dopamine D2 receptors (Fig. 1). Carbon-11-NMB cleared twelve times faster from the frontal cortex and four times faster from the cerebellum than NMSP (*15*).

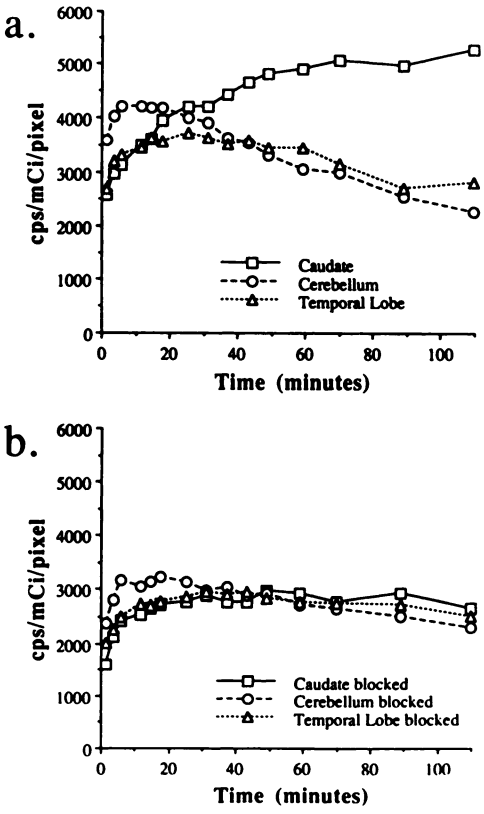

FIGURE 6
Time-activity curves in baboon caudates, temporal cortex, and cerebellum after injection of ^{11}C-NMB without (a) and with (b) preinjection of raclopride (2 mg/kg) at 5 min before radiotracer administration.

Carbon-11-NMB cleared significantly slower from the striatum than from all other regions (Fig. 1). As a result, a high ratio between striatum and the other regions of 11:1 was reached as early as 60 min after injection (Fig. 2). The peak striatal-to-cerebellar ratio for ^{11}C-NMB was six times higher than ^{77}Br-brombenperidol (*20*) and twice as high as ^{3}H- and ^{18}F-spiperone (*15,18*).

The high in vivo selectivity of ^{11}C-NMB to the dopamine D2 receptor, which was strongly indicated by the in vivo kinetics in different areas of the CNS (Fig. 1), was confirmed by the blocking experiments. Neither serotonin S2 or α-adrenergic receptor antagonists had any significant effect on the ^{11}C-NMB binding in the regions abundant in dopamine D2 receptors (Fig. 3). In vitro data suggest that N-methylation of benperidol resulted in decreased affinity for both the dopamine D2 and serotonin S2 receptors. However, larger effects were observed on the affinity for the serotonin S2 receptor than for the dopamine D2 receptor. The affinity of NMB for the dopamine D2 receptor was 4–5 times lower than that of benperidol and 20 times lower than that of NMSP (*34*), whereas its affinity for the serotonin S2 was one order of magnitude lower than that of benperidol and two orders of magnitude lower than that of NMSP. Therefore, the high selectivity of ^{11}C-NMB shown in vivo was most likely due to a weak interaction with the serotonin S2 receptor and/or a low incidence of rebinding of this compound to receptor sites, and consequently, a rapid washout from the S2 receptor-rich tissues (*35*).

Blocking experiments confirmed that the long retention of ^{11}C-NMB in the striatum was the result of interaction with the dopamine D2 receptor (Figs. 3, 5, and 6b). With haloperidol and raclopride, both highly specific dopamine D2 receptor ligands, at concentrations of 1000 times the mass dose of NMB, 88%–99% of the ^{11}C-NMB binding sites in the striatum were blocked. In the baboon brain, raclopride almost completely blocked the binding sites for ^{11}C-NMB (Figs. 5 and 6b) at a concentration 2000 times the mass dose of NMB. Therefore, it can be concluded that ^{11}C-NMB labels the dopamine D2 receptor site. In contrast, the ^{11}C-NMSP binding was not inhibited completely by a 1000–2000 times excess amount of unlabeled NMB, a butyrophenone ligand similar to spiperone, or raclopride, an example of a different class of dopamine D2 receptor ligands (Fig. 4). After blockade by these ligands, 30%–45% of the binding sites for ^{11}C-NMSP remained unblocked. A very high dose of raclopride (e.g., 10 mg/kg or 29 μmole/kg) blocked 83% of the ^{11}C-NMSP binding sites. However, with this dose of

raclopride not only D2-specific binding sites but also nonspecific sites (*36*) would possibly be blocked. These data are in agreement with results by other investigators who performed in vivo and in vitro inhibition studies with ^{3}H-spiperone or ^{3}H-NMSP (*1,29,37,38*). Together, the findings of this study and that of others suggest that NMSP and spiperone have multiple binding sites in dopamine D2 receptor-rich regions; some of which are not shared with NMB, haloperidol, or raclopride.

The 20% blockade of ^{11}C-NMB binding in the striatum by 1 mg/kg of SCH-23390, a dopamine D1-specific antagonist, might suggest an interaction of this compound with dopamine D1 receptors sites. Similarly, a previous study from this laboratory indicated that ^{11}C-SCH-23390 binding in the striatum is blocked to the same degree by 1 mg/kg of haloperidol (*39*). These findings considered together might indicate that dopamine D1 and dopamine D2 receptor sites are linked. A pharmacologic linkage between these two different types of dopaminergic receptors has been suggested by Parashos et al. (*40*).

In contrast to ^{11}C-NMB, ^{11}C-NMSP binding in serotonin S2 receptor-rich areas of the mouse brain was considerably higher (6 times in frontal cortex, 3 times in the rest of cortex) (Fig. 4). No significant blocking effects on ^{11}C-NMSP binding in these tissues in the presence of unlabeled NMB were observed, suggesting that NMB and NMSP have no common binding sites in cortical areas.

As in the mouse brain, highly selective in vivo labeling with ^{11}C-NMB was observed in the baboon (Fig. 5). The ratio between cortical regions and the cerebellum remained almost unity throughout the observation period (Fig. 6a). For comparison, the frontal cortex-to-cerebellar ratio for another highly selectively tracer, ^{18}F-fluoro-ethylspiperone (*4*), was ~1.3 at 90 min post-administration.

The ^{11}C-NMB specific binding in the striatum is reversible (Fig. 1) in contrast to the binding of ^{11}C-NMSP, ^{18}F-NMSP, or ^{18}F-spiperone. The biologic half-life of the ^{11}C-NMB specific binding in mice was much shorter (57 min) than that of ^{3}H-NMSP (20 hr) and ^{3}H-spiperone (30 hr) (*15*). Since reversibility is one of the characteristics of a receptor-ligand interaction, the high reversibility observed for ^{11}C-NMB may be an advantage of using this ligand for PET receptor studies. With both ^{18}F-NMSP or ^{18}F-spiperone, the binding to the dopamine D2 receptor is essentially irreversible during the observation time (4–9 hr) (*3,19*) and a dissociation of the ligand from the receptor sites could not be observed. The reversibility of ^{11}C-NMB binding to the dopamine D2 receptor, however, was not obvious in the baboon brain (Fig. 6a). This may be due to an equilibrium which was reached rather late and the subsequent clearance could not be examined adequately because of the short physical half-life of ^{11}C.

In conclusion, ^{11}C-NMB has excellent properties as an in vivo dopamine D2 receptor radiotracer. It gives high specific-to-nonspecific binding ratios and the in vivo binding is highly specific and selective for the dopamine D2 receptor.

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