

Evaluation of ^{11}C -Me-NB1 as a Potential PET Radioligand for Measuring GluN2B-Containing NMDA Receptors, Drug Occupancy, and Receptor Cross Talk

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Clinical and preclinical research with modulators at the *N*-methyl-D-aspartate (NMDA) receptor GluN2B N-terminal domain (NTD) aims for the treatment of various neurologic diseases. The interpretation of the results is hampered by the lack of a suitable NMDA PET tracer for assessing the receptor occupancy of potential drugs. We have developed ^{11}C -Me-NB1 as a PET tracer for imaging GluN1/GluN2B-containing NMDA receptors and used it to investigate in rats the dose-dependent receptor occupancy of eliprodil, a GluN2B NTD modulator. **Methods:** ^{11}C -Me-NB1 was synthesized and characterized by in vitro displacement binding experiments with rat brain membranes, in vitro autoradiography, and blocking and displacement experiments by PET and PET kinetic modeling. Receptor occupancy by eliprodil was studied by PET with ^{11}C -Me-NB1. **Results:** ^{11}C -Me-NB1 was synthesized at 290 ± 90 GBq/ μmol molar activity, 7.4 ± 1.9 GBq total activity at the end of synthesis ($n = 17$), and more than 99% radiochemical purity. ^{11}C -Me-NB1 binding in rat brain was blocked in vitro and in vivo by the NTD modulators Ro-25-6981 and eliprodil. Half-maximal receptor occupancy by eliprodil occurred at $1.5 \mu\text{g}/\text{kg}$. At $1 \text{ mg}/\text{kg}$ of eliprodil, a dose with reported neuroprotective effects, more than 99.5% of binding sites were occupied. In vitro, ^{11}C -Me-NB1 binding was independent of the σ -1 receptor (Sigma1R), and the Sigma1R agonist (+)-pentazocine did not compete for high-affinity binding. In vivo, a $2.5 \text{ mg}/\text{kg}$ dose of (+)-pentazocine abolished ^{11}C -Me-NB1-specific binding, indicating an indirect effect of Sigma1R on ^{11}C -Me-NB1 binding. **Conclusion:** ^{11}C -Me-NB1 is suitable for the in vivo imaging of NMDA GluN1/GluN2B receptors and the assessment of receptor occupancy by NTD modulators. GluN1/GluN2B NMDA receptors are fully occupied at neuroprotective doses of eliprodil. Furthermore, ^{11}C -Me-NB1 enables imaging of GluN1/GluN2B NMDA receptor cross talk.

Key Words: NMDA; PET; receptor occupancy; eliprodil; NTD modulator

J Nucl Med 2018; 59:698–703
DOI: 10.2967/jnumed.117.200451

Pharmacologic modulation of *N*-methyl-D-aspartate (NMDA) receptors may become an efficient therapeutic strategy for the treatment of cerebral ischemia, Alzheimer disease, Huntington disease, Parkinson disease, depression, neuropathic pain, and schizophrenia (1). A functional NMDA receptor consists of 4 subunits of the 3 subfamilies GluN1, GluN2A–D, and GluN3A,B. Typically, 2 subunits are GluN1 and the other 2 are mainly from the GluN2 subfamily (1,2). The receptor is coactivated by glutamate and glycine or D-serine. Besides the agonist binding domains, the receptor offers additional sites for pharmacologic modulation (1,2). Ifenprodil, eliprodil, and Ro-25-6981 bind to the N-terminal domain (NTD) interface of 2 subunits with high selectivity for GluN1/GluN2B (NTD binding site) over GluN1/GluN2A (1,2). Despite promising in vitro results with NTD modulators, effective doses in preclinical studies were often higher than expected, and results from clinical trials did not meet the expectations (3,4). Ligand sharing between the NTD binding site and σ -receptors, and the direct or indirect control of NMDA receptor activity and localization by the σ -1 receptor (Sigma1R) and dopamine receptors, are among the discussed confounding factors (5,6).

In our efforts to develop a PET radioligand for imaging GluN1/GluN2B receptors, we developed ^{11}C -Me-NB1 (Fig. 1). ^{11}C -Me-NB1 is based on WMS-1405 (herein designated Me-NB1), an NTD binding site ligand derived from ifenprodil with improved metabolic stability, 5.4 nM affinity (K_i) to hGluN1/hGluN2B, and no modulatory effect at hGluN1/hGluN2A receptors. It has 33- and 100-fold selectivity for hGluN1/hGluN2B over human Sigma1R and σ -2 receptors (Sigma2R), respectively (7). Applying ^{11}C -Me-NB1, we characterized the distribution pattern of NTD modulator binding sites in the rat brain and investigated their dose-dependent occupancy by eliprodil ($K_i = 13$ nM for hGluN1/hGluN2B; $K_i = 132$ and 634 nM for rat Sigma1R and Sigma2R, respectively) (8). Furthermore, we examined whether ^{11}C -Me-NB1 PET can be used to investigate receptor cross talk involving GluN1/GluN2B receptors.

MATERIALS AND METHODS

Radiolabeling and In Vitro Characterization

^{11}C -Me-NB1 was produced by reacting ^{11}C -CH₃I with 3-(4-phenylbutyl)-2,3,4,5-tetrahydro-1*H*-3-benzazepine-1,7-diol (9) (Fig. 1). The log *D* was determined by the shake-flask method with ^{11}C -Me-NB1 in *n*-octanol/phosphate-buffered saline, pH 7.4. Binding affinities were determined in competition binding assays with rat brain membranes

Received Aug. 4, 2017; revision accepted Sep. 30, 2017.

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Published online Nov. 30, 2017.

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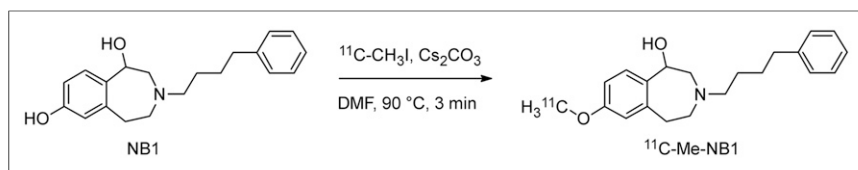


FIGURE 1. Radiosynthesis of ^{11}C -Me-NB1. DMF = dimethylformamide.

(10–12). For autoradiography, horizontal brain slices of adult male wild-type mice and Wistar rats or Sigma1R knockout mice lacking the Sigma1R encoding genomic sequence except for the first 4 codons (13) were incubated with ^{11}C -Me-NB1 alone or in combination.

Ex Vivo Biodistribution

Animal experiments were in accordance with the Swiss legislation on animal welfare and approved by the Veterinary Office of the Canton Zurich, Switzerland. Twelve conscious male Wistar rats (296–373 g) received, via the tail vein, a 2 mL/kg injection of eliprodil vehicle (5% glucose, 0.45% NaCl, 1 mM citric acid, pH 7) or a 2 mg/kg injection of eliprodil (1 mg/mL) and, 1 min later, 3.1–36.3 MBq (0.5–1.6 nmol/kg) of ^{11}C -Me-NB1 in 250 μL of saline containing no more than 8% ethanol. The rats were sacrificed by decapitation under isoflurane anesthesia 15 min after tracer injection. Tissues were dissected and weighed, radioactivity was quantified, and SUVs were calculated (Supplemental Fig. 1; supplemental materials are available at <http://jnm.snmjournals.org>).

PET Experiments with an Input Function

Seven male Wistar rats (331–353 g) were scanned with simultaneous recording of the blood coincidences (14). In brief, anesthesia was initiated with 5% isoflurane 40–60 min before the start of the scan and maintained at 2.5%–5% isoflurane. An arteriovenous shunt was applied by cannulation of the tail artery and a lateral tail vein. The rat was transferred to the scanner bed (SuperArgus PET/CT scanner [Sedecal]; axial field of view, 4.8 cm; spatial resolution, 1.6–1.7 mm in full width at half maximum (15)), and the shunt was guided through a Twilite coincidence counter (Swisstrace) and a peristaltic pump (200–300 $\mu\text{L}/\text{min}$). The blood counter and PET scanner were started, and at time zero, 32–52 MBq (0.44–0.78 nmol/kg, 250–300 μL) of ^{11}C -Me-NB1 were injected via a separate, cannulated tail vein. Data were acquired for 60 min, followed by a CT scan. The rats were euthanized under anesthesia immediately after the scan. Between 28 and 31 min before the start of the scan, the rats received either a 25 mg/kg intraperitoneal injection of ketamine in water (25 mg/mL; 3 rats) or water alone (1 mL/kg; 4 rats). Only 2 (with ketamine) and 3 (baseline) blood curves were available for analysis, because the arteriovenous shunt became clogged in 2 rats. The ratios of parent tracer to total radioactivity in plasma and homogenized brain were determined from additional rats. The plasma-to-whole-blood radioactivity ratio was 1.11.

PET Experiments Without an Input Function

Twenty-three Wistar rats (327–409 g) were scanned as described above but without an arteriovenous shunt. Anesthesia was induced 15–35 min before tracer injection. Test compounds in less than 1 mL of vehicle (Me-NB1, the same as eliprodil; (+)-pentazocine, 2:8 0.1 M HCl:lactated Ringer solution; Ro-25-6981, 1:1 polyethylene glycol 300:saline) were injected intravenously on the scanner bed under anesthesia at 1 min (Me-NB1, eliprodil, and (+)-pentazocine), 5 min (Ro-25-6981), or 20 min (haloperidol (16)) before ^{11}C -Me-NB1. The injected doses of ^{11}C -Me-NB1 were 20–89 MBq. The total doses were 0.4–3,000 nmol/kg (0.00013–1 mg/kg) for ^{11}C -Me-NB1 and Me-NB1, 0.7–5,700 nmol/kg (0.00025–2 mg/kg) for eliprodil, 16.4 $\mu\text{mol}/\text{kg}$

(7.5 mg/kg, free base) for Ro-25-6981, 346 nmol/kg (0.13 mg/kg) for haloperidol (Haldol; Janssen-Cilag), and 8.8 $\mu\text{mol}/\text{kg}$ (2.5 mg/kg) for (+)-pentazocine. For baseline scans, the respective vehicle was injected. In a displacement experiment, eliprodil, 1 mg/kg, in 450 μL of vehicle was injected into the tail vein 20 min after the start of the scan.

PET Data Reconstruction and Analysis

PET data were reconstructed as described in Supplemental Figure 1. Images and time-activity curves were generated with PMOD, version 3.7 (PMOD Technologies), with predefined regions of interest, modified from the PMOD template (Supplemental Fig. 1). Scans with an input function were analyzed with Matlab (MathWorks) by compartmental modeling (14,17). In brief, the clearance parameter of tracer transfer from plasma to brain (K_1), the rate constants of transfer out of the brain (k_2), receptor binding (k_3), and dissociation from the receptor (k_4), as well as the K_1 and k_2 of the radiometabolite (K_{1M} , k_{2M}), were fitted for whole brain for the full scan duration, and the respective volume of distribution (V) of total tracer ($V_{T,\text{fit}} = K_1/k_2 \times (1 + k_3/k_4)$), the V of specifically bound tracer ($V_S = K_1/k_2 \times k_3/k_4$), the V of nondisplaceable tracer ($V_{ND} = K_1/k_2$), the V ratio V_T/V_{ND} (DVR), and the nondisplaceable binding potential ($\text{BP}_{ND} = k_3/k_4 = \text{DVR} - 1$) were calculated (17,18). V_T was also determined from the Logan plot ($V_{T,\text{Logan}}$) (17,19). Blood coincidence start time and background, and a biexponential function describing the ratio of parent to total radioactivity in plasma, were fitted together with the rate constants. The results were confirmed with the PKIN module of PMOD, version 3.9. For the fits of the individual brain regions and truncated scan durations, only K_1 to k_4 were fitted; all other parameters were used as fitted for whole brain.

For quantification without an input function, SUV was averaged from 0 to 60 min ($\text{SUV}_{0-60\text{min}}$). For the receptor occupancy study, $\text{SUV}_{0-60\text{min}}$ was fitted with the function in Equation 1.

$$\text{SUV}_{0-60\text{min}} = (\text{SUV}_{0-60\text{min},\text{max}} - \text{SUV}_{0-60\text{min},\text{min}}) \times D_{50}/(D_{50} + d) + \text{SUV}_{0-60\text{min},\text{min}} \quad \text{Eq. 1}$$

$\text{SUV}_{0-60\text{min},\text{min}}$ and $\text{SUV}_{0-60\text{min},\text{max}}$ are the plateaus, and d is the sum of the eliprodil and the ^{11}C -Me-NB1 and Me-NB1 doses, with the latter multiplied by a weighting factor f . The fitted parameters were dose at half maximal receptor occupancy (D_{50}), f , $\text{SUV}_{0-60\text{min},\text{min}}$, and $\text{SUV}_{0-60\text{min},\text{max}}$. Receptor occupancy ($d/(D_{50} + d)$) was calculated by rearranging Equation 1. Percentage specific binding was calculated as $(\text{SUV}_{0-60\text{min},\text{max}} - \text{SUV}_{0-60\text{min},\text{min}})/\text{SUV}_{0-60\text{min},\text{max}} \times 100\%$.

Statistical Analysis

Mean values are shown with SD and compared by a homoscedastic, 2-tailed Student t test (without correction for multiple comparisons). The Akaike information criterion, corrected for finite sample size, was calculated according to a previously published method (20).

RESULTS

Tracer Radiosynthesis and In Vitro Characterization

^{11}C was radiolabeled by reacting the phenolic precursor with ^{11}C -MeI to afford ^{11}C -Me-NB1 (Fig. 1) in $290 \pm 90 \text{ GBq}/\mu\text{mol}$ molar activity and $7.4 \pm 1.9 \text{ GBq}$ total activity at the end of synthesis ($n = 17$). Radiochemical purity was more than 99%. Total synthesis time from end of bombardment was 35–40 min. Log D at pH 7.4 was 1.4 ± 0.2 ($n = 3$). In the in vitro competition binding assay, Ro-25-6981 (7.2 nM affinity to the human NTD

binding site; similar affinity to guinea pig Sigma1R and Sigma2R (21,22)) competed with the binding of both ^3H -ifenprodil and the Sigma1R agonist (+)- ^3H -pentazocine with 2-digit nanomolar K_i (Supplemental Table 1). Haloperidol, an antagonist for D2, D3, and D4 receptors and an inverse agonist of Sigma1R (7,23), competed in the micromolar range with ^3H -ifenprodil (20°C and 37°C) and (+)- ^3H -pentazocine (20°C) but with 2-digit nanomolar K_i with (+)- ^3H -pentazocine at 37°C. The K_i for the competition of eliprodil with ^3H -ifenprodil was 48.5 ± 32.0 nM (20°C), in agreement with previous findings (4). Its competition with (+)- ^3H -pentazocine was weak ($K_i \geq 229$ nM, 37°C). Me-NB1 bound with a K_i of 40.2 ± 2.8 nM in competition with ^3H -ifenprodil but competed only weakly with (+)- ^3H -pentazocine (half-maximal inhibitory concentration > 4 μM , 37°C), confirming a high selectivity of Me-NB1 for the rat NTD binding site over rat Sigma1R.

^{11}C -Me-NB1 Autoradiography and Biodistribution

In vitro autoradiography revealed binding of ^{11}C -Me-NB1 throughout the brain. Binding was reduced by eliprodil (54 nM, 10 μM), Ro-25-6981 (100 μM), haloperidol (10 μM), or an excess of Me-NB1 (100 μM) but not by 1 mM glutamate (Supplemental Fig. 2). Specific binding was confirmed in ex vivo biodistribution experiments (Supplemental Fig. 3). Brain SUVs were 3.8 ± 1.2 for midbrain, 3.7 ± 1.0 for cortex, 2.8 ± 0.8 for cerebellum, and 2.7 ± 0.7 for olfactory bulb ($n = 8$). Eliprodil (2 mg/kg intravenously) reduced ^{11}C -Me-NB1 accumulation in all brain regions. The relative reduction was highest for midbrain, brain stem, cortex, and striatum (all $> 40\%$ and significant) and lowest and not significant for hippocampus (31.9%) and cerebellum (34.5%). In peripheral tissues, accumulation was highest in adrenal glands (SUV, 10.3 ± 2.9 ; 52.9% specific). The SUV in blood was significantly ($P = 0.002$) higher under blocking conditions (0.300 ± 0.035) than under baseline conditions (0.194 ± 0.045).

^{11}C -Me-NB1 PET and PET Kinetic Modeling

Figure 2 shows PET images under baseline and blocking conditions. Time-activity curves of ^{11}C -Me-NB1 in various brain regions and after blocking and displacement with eliprodil are shown in Figure 2 and Supplemental Figure 4. Both blocking and displacement reduced the SUV, further confirming the specific and reversible binding of ^{11}C -Me-NB1.

Data of 5 scans with an arterial blood time-activity curve were available, and brain time-activity curves were fitted to a 1- and 2-tissue-compartment model. The presence of a polar radiometabolite in plasma and up to 7% in brain (determined from brain homogenates) was considered for the analysis (Fig. 3). Fits with a 2-tissue-compartment model and the respective Logan plots are shown in Figure 3 (whole brain) and Supplemental Figures 5 and 6 (regions). Parametric maps for V_T for the

scan in Figure 2A are shown in Supplemental Figure 7. $V_{T,\text{fit}}$ and $V_{T,\text{Logan}}$ of 12 brain regions each for the 5 scans are depicted in Supplemental Figure 8. Supplemental Figure 9 shows the fit of the data in Figure 3 with a 1-tissue-compartment model and the residuals for both models. The Akaike information criterion favored the 2-tissue-compartment model. For all scans, $V_{T,\text{fit}}$ of whole brain remained within $\pm 5\%$ when the scan end time for data analysis was reduced to any time between 13 and 60 min (Fig. 3D). Ignoring radiometabolite distribution to the brain resulted in similar fit parameters. Supplemental Tables 2–4 show the fit parameters for whole brain on the individual scans. Averaged for the 5 scans and for whole brain, $V_{T,\text{fit}}$ was 11.8 ± 0.7 ; BP_{ND} , 3.77 ± 0.97 ; and V_{ND} , 2.57 ± 0.56 . Average K_1 was 2.9 ± 0.9 mL/cm 3 /min; k_2 , 1.2 ± 0.6 min $^{-1}$; k_3 , 0.73 ± 0.09 min $^{-1}$, and k_4 , 0.20 ± 0.04 min $^{-1}$.

PET Quantification Without an Arterial Input Function and Effect of Ketamine

Because a reference region without specific binding of ^{11}C -Me-NB1 was not available for input-function independent analysis (17), $\text{SUV}_{0-60\text{min}}$ was evaluated as a surrogate for V_T . The Pearson r^2 for the linear correlation between $\text{SUV}_{0-60\text{min}}$ and $V_{T,\text{fit}}$ of 5 rats (12 brain regions) was 0.913 (Supplemental Fig. 8B). The transformed (according to the correlation) $\text{SUV}_{0-60\text{min}}$ of all 4 baseline scans and 3 scans after ketamine challenge agreed with $V_{T,\text{fit}}$ values (Supplemental Fig. 10). The subtherapeutic dose of ketamine was administered to investigate whether changes in glutamate

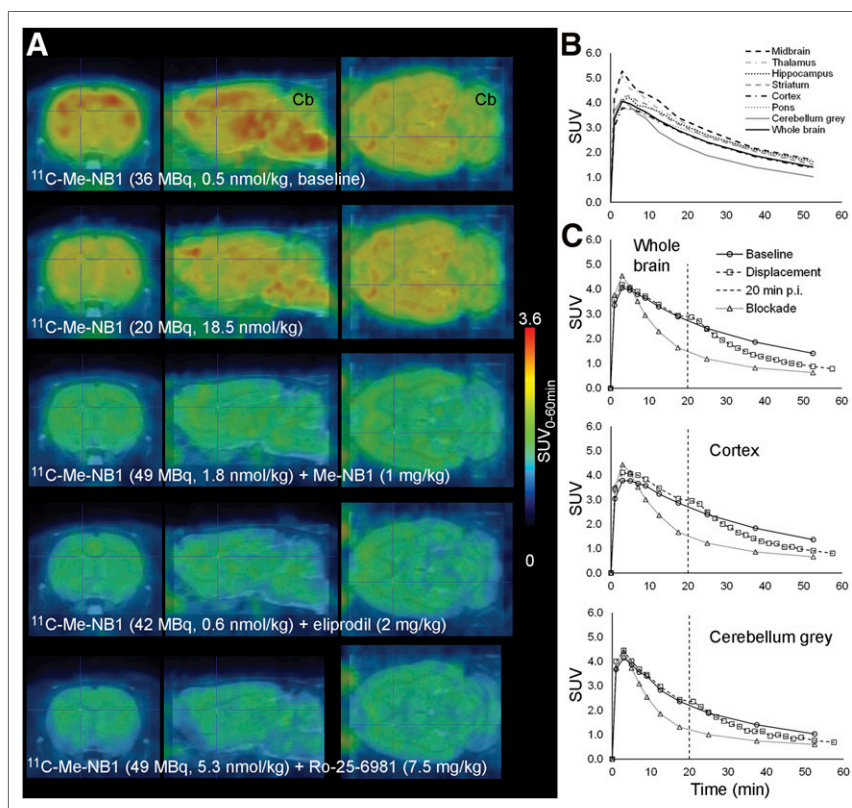


FIGURE 2. (A) Rat brain ^{11}C -Me-NB1 PET images (0–60 min) superimposed on MRI template (PMOD). (B) Brain region time-activity curves of baseline scan (67 MBq, 2.1 nmol/kg, 394 g). (C) Time-activity curves under baseline conditions (as in B) or with 1 mg of intravenous eliprodil per kilogram 1 min before ^{11}C -Me-NB1 (blockade: 35 MBq, 0.44 nmol/kg, 370 g) or 20 min after ^{11}C -Me-NB1 injection (displacement: 55 MBq, 0.57 nmol/kg, 449 g). Vertical dashed line indicates time point of eliprodil intravenous injection for displacement. Cb = cerebellum.

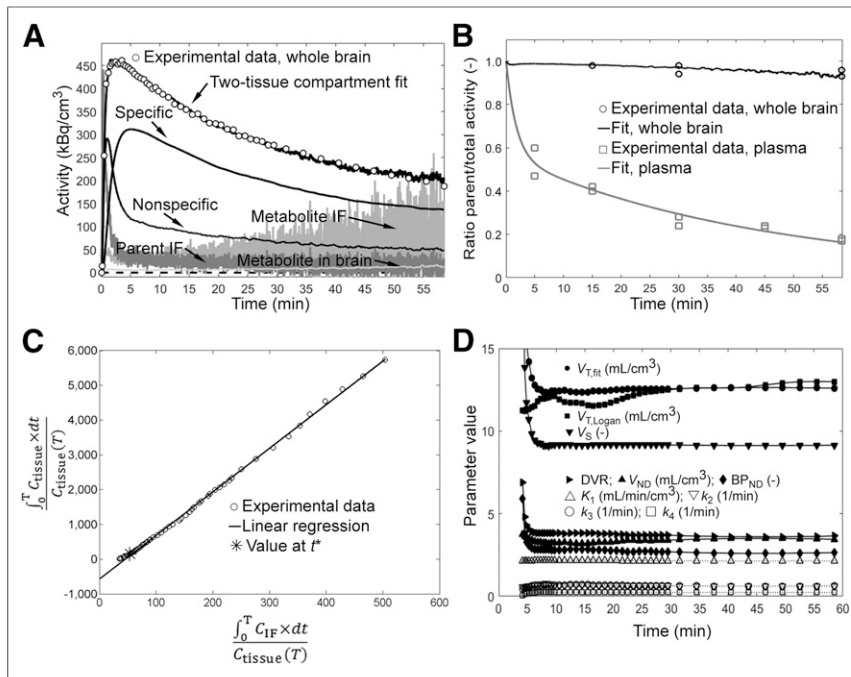


FIGURE 3. ^{11}C -Me-NB1 kinetic evaluation in rat whole brain for representative baseline scan (31.8 MBq, 0.44 nmol/kg, 321 g). (A) Whole-brain time-activity curve ($\circ$) and input functions (IF). Black lines indicate fit results, and 100 kBq/cm³ corresponds to ~ 0.4 nM ^{11}C -Me-NB1. $V_{T,\text{fit}} = 12.6$ mL/cm³. (B) Experimental and fit ratios of parent tracer to total radioactivity in plasma and brain. Residuals from experimental time-activity curve and parent-to-total-radioactivity ratios were minimized simultaneously during fitting procedure. (C) Logan plot. Plasma and brain time-activity curves were corrected for the fitted parent-tracer-to-total-radioactivity ratios. $V_{T,\text{Logan}} = 12.5$ mL/cm³. Linear regression of data is for $t > 3$ min. $C_{\text{tissue}} =$ radioactivity in tissue (image data); $C_{\text{IF}} =$ radioactivity in arterial plasma; $T =$ time points. (D) Fit parameters for scan durations of between 4 and 60 min.

levels affect ^{11}C -Me-NB1 accumulation (24). Our results (V_T and $\text{SUV}_{0-60\text{min}}$) excluded a major influence of ketamine on ^{11}C -Me-NB1 accumulation under isoflurane anesthesia (Supplemental Fig. 10; Supplemental Tables 3 and 4).

Receptor Occupancy by Eliprodil

Using ^{11}C -Me-NB1, we determined the dose-dependent occupancy of the NTD binding sites by eliprodil. D_{50} was 4.2 nmol/kg (1.5 $\mu\text{g/kg}$) for whole brain (Fig. 4). The fit dose to reach 99% occupancy was 150 $\mu\text{g/kg}$. Specific binding was 46.9%. Fits for individual brain regions revealed D_{50} values of between 3.2 and 7.3 nmol/kg (Supplemental Fig. 11). Nondisplaceable tracer uptake had no effect on D_{50} (Supplemental Fig. 12). A Lassen plot analysis (25) revealed a D_{50} of 8.5 nmol/kg (Supplemental Fig. 13).

Imaging Receptor Cross Talk with ^{11}C -Me-NB1

NMDA receptor activity is modulated by Sigma1R and dopamine receptors (6,26,27). We used (+)-pentazocine and haloperidol to modulate Sigma1R and dopamine receptors, respectively. Both (+)-pentazocine and haloperidol showed only weak competition with ^3H -ifenprodil in the competition binding assays. (+)-pentazocine brain concentrations

were aimed at 350–2,000 nM (28), and the dose of haloperidol corresponded to 10-fold the D_{50} in a D_2 and D_3 receptor occupancy study with ^{11}C -raclopride in rats (16). At these concentrations, Sigma1Rs and D_2 and D_3 receptors, respectively, should be occupied, whereas no direct binding to GluN1/GluN2B receptors should occur. Both haloperidol and (+)-pentazocine abolished ^{11}C -Me-NB1-specific binding under these conditions (Fig. 5A).

To exclude both a direct competition between ^{11}C -Me-NB1 and (+)-pentazocine binding, and high-affinity binding of ^{11}C -Me-NB1 to Sigma1R, we performed in vitro autoradiography with brain slices from Sigma1R knockout mice (Figs. 5E and 5F and Supplemental Fig. 14). ^{11}C -Me-NB1 binding did not differ between slices from wild-type and Sigma1R knockout mice, excluding significant binding of ^{11}C -Me-NB1 to Sigma1R. (+)-pentazocine (1 μM) reduced the binding for both genotypes by less than 10% ($P < 0.05$), whereas the same concentration of eliprodil reduced the binding for both genotypes by 40% ($P < 0.01$), similar to the ex vivo biodistribution experiment and receptor occupancy study. Autoradiography at 3 different ^{11}C -Me-NB1 concentrations revealed high-affinity binding in all brain regions of rat, mouse, and Sigma1R knockout mouse, including the GluN2B-free cerebellum, in agreement with the PET results.

DISCUSSION

To date, no clinically validated PET radioligand exists for the imaging of NMDA receptors. Reasons for failure include lack of in vivo specificity and selectivity, low affinity, poor metabolic stability, and poor distribution to the brain (29–34). Aiming to overcome the shortcomings of previously reported radioligands, and given the excellent in vitro properties of Me-NB1, we

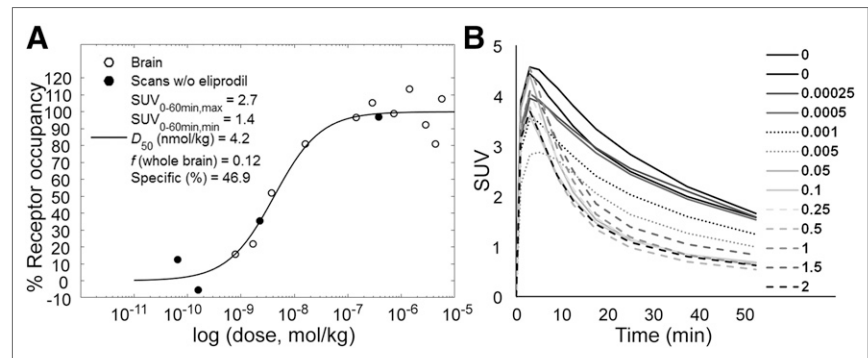


FIGURE 4. (A) Rat whole-brain NTD binding site occupancy by eliprodil. $\circ = ^{11}\text{C}$ -Me-NB1 with eliprodil; $\bullet = ^{11}\text{C}$ -Me-NB1/Me-NB1 alone. Receptor occupancy by ^{11}C -Me-NB1 and Me-NB1 was included in saturation function. $\text{SUV}_{0-60\text{min}}$ was fitted to saturation function, and data were transformed to percentage receptor occupancy. Solid line indicates transformed fit. (B) Respective time-activity curves, with eliprodil doses indicated in mg/kg.

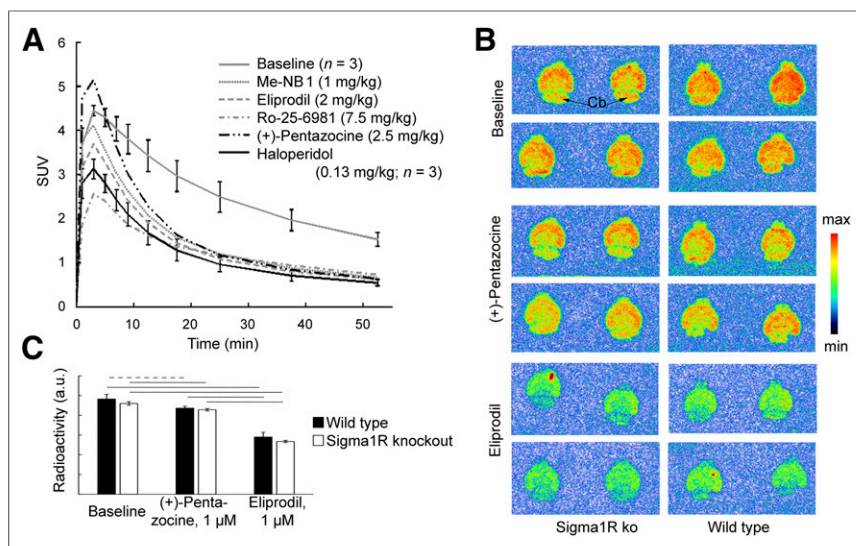


FIGURE 5. Influence of (+)-pentazocine and haloperidol on ^{11}C -Me-NB1 PET in rat brain (A), and in vitro autoradiography with brain slices of S1R knockout (ko) mice (B and C). (A) Time-activity curves after drug administration and after blocking with Me-NB1, eliprodil, and Ro-25-6981 are shown. SUV within first few minutes may be affected by drug-induced changes in cerebral blood flow (46,47) and increased plasma levels under blocking conditions (Fig. 3). (B) ^{11}C -Me-NB1 autoradiograms of brain slices from Sigma1R knockout and wild-type mice. Incubation was with 4.0 nM ^{11}C -Me-NB1 alone (baseline) or together with (+)-pentazocine or eliprodil. (C) Quantitative analysis of autoradiograms in B. No significant difference was found between wild-type and Sigma1R knockout mice for any of the 3 conditions ($P > 0.05$). (+)-pentazocine (1 μM) reduced ^{11}C -Me-NB1 binding in wild-type and Sigma1R knockout mice by $<10\%$. Reduction by equal concentration of eliprodil was 40% and 42%, respectively (horizontal dashed line, $P < 0.05$; solid lines, $P < 0.01$). a.u. = arbitrary units.

developed ^{11}C -Me-NB1 for imaging GluN2B receptors. ^{11}C -Me-NB1 was obtained in high radiochemical yields and molar activities. In vivo, it followed a 2-tissue-compartment model, with $\text{SUV}_{0-60\text{min}}$ as a useful surrogate for V_T , but with the caveat of its inherent high variability (17).

^{11}C -Me-NB1 binding faced competition from low doses of eliprodil in all brain regions, including midbrain, pons, and cerebellum with low GluN2B levels (35–38). Off-target binding to Sigma1R was excluded in vitro. The GluN2B-poor regions contain significant levels of GluN2D or GluN2C (36). ^{11}C -Me-NB1 and eliprodil may bind to these or alternative GluN receptor subtypes in addition to GluN1/GluN2B receptors. The high specific binding of ^{11}C -Me-NB1 in adrenal gland tissue, which contains GluN1, GluN2C, and GluN2D but not GluN2A or GluN2B (39), supports this hypothesis.

In preclinical studies, eliprodil had neuroprotective effects at 1 mg/kg (4,40,41). This corresponds to full ($\geq 99.5\%$) receptor occupancy in our study. As discussed by Carter et al. (4), antidepressant and anticonvulsive effects observed at even higher doses may have resulted from lower-affinity binding to alternative targets.

The Sigma1R agonist (+)-pentazocine abolished ^{11}C -Me-NB1-specific binding in the rat brain despite the lack of direct competition in vitro. (+)-pentazocine stabilizes the Sigma1R in its dimeric and monomeric forms, which interact with other proteins (23). Besides indirect effects on NMDA receptors, such as phosphorylation, a direct interaction between Sigma1R and the NTD of GluN1 that is involved in the interface with the GluN2B NTD was recently demonstrated (42). This interaction may directly hamper ^{11}C -Me-NB1 binding. Alternatively, direct or indirect NMDA

receptor modulation could bring the receptor to a conformation with reduced binding affinity for NTD ligands. Finally, altered receptor trafficking and cell-surface density could affect ^{11}C -Me-NB1 accumulation.

Several mechanisms could result in the elimination of ^{11}C -Me-NB1-specific binding observed with haloperidol. Activation of dopamine receptors modulates NMDA receptor-mediated Ca^{2+} currents, and treatment of rats with a 1 mg/kg dose of haloperidol altered the phosphorylation pattern of GluN1 and GluN2B (6,26,43,44). Haloperidol also modulates σ -receptors (7,45). We did not further investigate the molecular mechanisms. However, our data suggest that ^{11}C -Me-NB1 PET could become a useful tool to visualize the consequences of receptor cross talk involving GluN2B-containing NMDA receptors.

CONCLUSION

We have successfully developed a PET tracer, ^{11}C -Me-NB1, that allows noninvasive imaging of the density of NTD binding sites containing NMDA receptors. In the future, this development may allow the density of GluN2B/GluN1A receptors in neuronal diseases to be followed noninvasively and receptor occupancy under

therapeutic intervention to be studied. Our receptor-occupancy study suggests that the published neuroprotective effects of eliprodil in rats required complete occupancy of the NTD binding sites. ^{11}C -Me-NB1 PET is sensitive to drug-induced receptor cross talk.

DISCLOSURE

This work was financially supported by the Swiss National Science Foundation (SNSF, grant 160403). No other potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

We thank Bruno Mancosu for producing tracer and Jose Miguel Vela Hernandez for providing us with Sigma1R $^{-/-}$ and wild-type mouse brain tissues.

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