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4,5,9,10-Tetrahydro-1,4-ethanobenz[*b*]quinolizine as a Prodrug for Its Quinolizinium Cation as a Ligand to the Open State of the TCP-Binding Site of NMDA Receptors

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Abstract—A new derivative of 4,5,9,10-tetrahydro-1,4-ethanobenz[*b*]quinolizine (**2**) has been designed as a prodrug for its quinolizinium cation (**1**) that is a potent antagonist of the TCP-binding site of NMDA receptors at the open state. The ^{11}C-labeled **2** showed high accumulation of radioactivity in the brain in an in vivo biodistribution study. The speculation of **2** as a prodrug of **1** has been proven by the fact that **1** was observed in a high ratio to **2** in an analysis by RP-HPLC of the brain homogenates.

The NMDA receptor is a member of ion channel-type receptors for L-glutamate, an excitatory amino acid in the mammalian central nervous system (CNS), and is characterized by activation with *N*-methyl-D-aspartate which increases the influx of Ca^{2+} through the ion channel of the receptor. NMDA-type glutamate receptors play a significant role in CNS functions such as synaptic transmission, learning and memory, and so on. Disorders of NMDA receptors may be included in ischeameic, hypoxic, hypoglycaemic, and traumatic insults epilepsy, AIDS dementia, and Huntington's and Alzheimer's diseases, and a number of studies have been performed to develop selective antagonists of NMDA receptors as therapeutic agents.[1]

Non-invasive imaging with the use of radio-labeled compounds such as those labeled with ^{18}F ($t_{1/2}$ = 109.7 min) or ^{11}C ($t_{1/2}$ = 20.3 min) of short half-life provides a useful and unique method, the so-called positron emission tomography (PET), for diagnosis of functions of the brain of a living human. This method has potential application to diagnosis of diseases with disorders of NMDA receptors with the use of selective radio-ligands for NMDA receptors. In spite of the fact that a number of radio-ligands have been derived from TCP, PCP, ketamine, or MK-801 and so on, representative antagonists at a site in the receptor-ion channel, selective in vivo biodistribution has not been achieved. Non-selective biodistribution is thought to be due to the hydrophobic nature of these ligands.[2] On the other hand, a new type of highly hydrophilic TCP-site ligands, 1,4-ethanobenzo[*b*]quinolizinium derivatives, has been developed as the specific ligands to the open state of the NMDA ion channel.[3] However, in our previous study, ^{11}C-labeled 1,4-ethanobenzo[*b*]quinolizinium derivative ([^{11}C]**1**) showed only a little accumulation in the brain in an in vivo study with mice.[4a] In the course of our continuing search for NMDA receptor specific radio-ligands, we designed the reduced derivative (**2**) as a prodrug for **1**. Here, we wish to report that the ^{11}C-labeled **2** showed high accumulation in the brain in an in vivo biodistribution study.

We found that the cationic ligand **1** was easily reduced to the corresponding neutral derivative (**2**), which was oxidized with dicyanodinitroquinone (DDQ) to reproduce

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1. Thus, we envisioned a pro-drug approach with the use of reduced derivative (**2**).[5] In the new approach, it was expected that a relatively hydrophobic ligand (**2**) would penetrate through the blood–brain barrier (BBB) and suffer in vivo oxidation in the brain to produce cationic ligand **1**, and finally **1** would remain in the brain (Fig. 1).

1,4-Ethanobenzo[*b*]quinolizinium (**5**) was obtained by Diels–Alder reaction with the use of benzo[*b*]quinolizinium (**3**) and dithiophene (**4**)[4b] (Scheme 1).[6] $NaBH_4$ reduction of **5** gave the reduced derivative (**6**).[7] Radiochemical synthesis was carried out in an automated synthesis apparatus for ^{11}C-labeled compound developed by Suzuki et al.[8] A mixture of **6** and [^{11}C]iodomethane in dimethylformamide was heated at 30 °C for 5 min in the presence of 2 equiv of NaH, and the mixture was purified by HPLC to provide [^{11}C]**2** in a synthesis time of 25 min from the end of the bombardment (EOB) (Scheme 2).

A radioactive peak corresponding to the retention time of authentic non-radiolabeled **2** was collected in more than 90% incorporation of radioactivity determined by HPLC of the ^{11}C-reaction mixture.[9] The specific activity of the product was estimated by UV spectroscopy to be >63 GBq/mmol. Radiochemical purity was greater than 98%. The purified ligand [^{11}C]**2** was used for the in vivo study without optical resolution. [^{14}C]**2** was synthesized in a similar manner with use of **6** and [^{14}C]iodomethane in dimethylformamide. The specific activity of the product was estimated by UV spectroscopy to be >0.3 MBq/μmol with radiochemical purity greater than 98%.

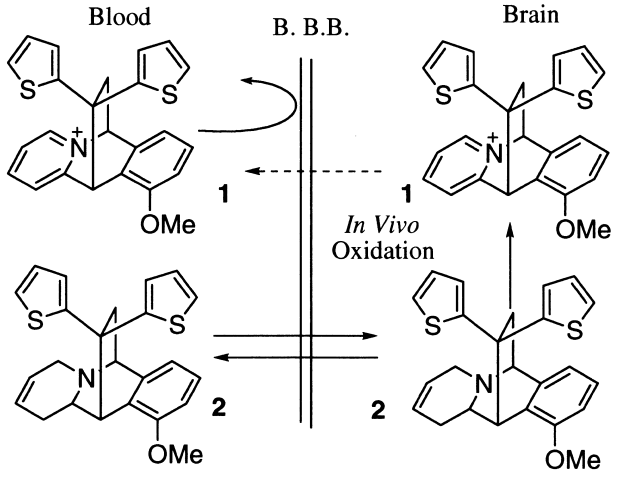
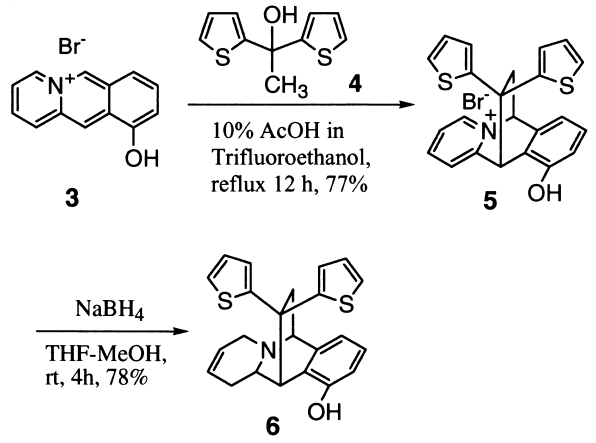
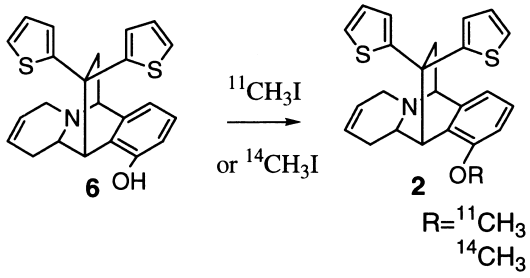


Figure 1. Design of tetrahydro derivative of **2** as a prodrug for **1**. After penetration into the brain, tetrahydro derivative **2** was expected to be oxidized in vivo to the cationic **1**, and retained in the brain.



Scheme 1.

In vivo oxidation of **2** was investigated with the use of ^{14}C-labeled **2**. A saline solution of [^{14}C]-labeled **2** was intravenously injected to ddY mice, and the mice were sacrificed at 15 min or 45 min after injection. The brain was homogenized with methanol, and centrifuged. The supernatant was passed through a filter, and injected to a RP-HPLC column.[10] Each fraction was collected every 30 s and its radioactivity was counted by a liquid scintillation counter. A peak corresponding to the cationic ligand **1** was clearly identified as the major peak together with unchanged **2** at 10.5 min after injection (Fig. 2). As **2** was almost unchanged in 0.1 M phosphate buffer (pH 7.4) at 37 °C and the cationic **1** showed only a little penetration into the brain,[4a] the formation of **1** should be indicative of oxidation of **2** in the mice brain. Thus, it has been proved that **2** can work as a prodrug for the cationic ligand **1**. It should be noted that **1** was cleared from the brain at 45 min after injection. The clearance of **1** from the brain might be mediated either by organic cation transporters or by metabolism of **1**.[11]



Scheme 2. (1) Radiosynthesis, $^{11}CH_3I$ (or $^{14}CH_3I$), NaH, DMF, 30 °C, 5 min; (2) HPLC purification, ODS, 85% MeOH/H_2O. Total synthesis time, 25 min.

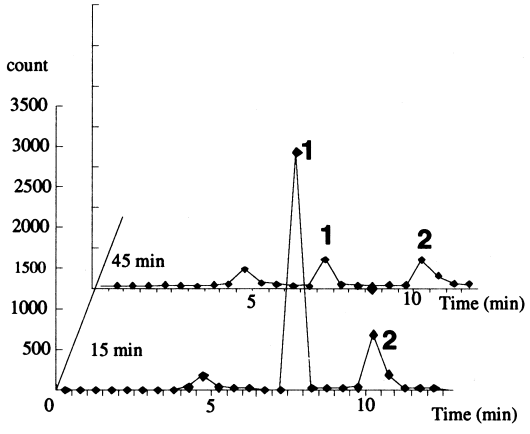


Figure 2. RP-HPLC analysis of the metabolite of **2** in mice brain homogenates 15 and 45 min after injection. The peak at around 5 min is unknown material, those marked with **1** and **2** are identical with those of non-labeled authentic samples.

The biodistribution of ^{11}C-labeled **2** was similarly investigated with ddY mice. Regional brain biodistribution of [^{11}C]**2** (5.1–6.8 MBq) following intravenous administration is summarized in Figure 3. In contrast to [^{11}C]**1** which showed only less than 0.1% dose/g,[4a] much higher accumulation of radioactivity in the brain was observed with [^{11}C]**2**. Radioactivity in the blood was cleared rapidly, whereas radioactivity in the brain was retained during the investigation period. The results that accumulations of radioactivity in the regional brain is higher than that in the blood after 15 min (Fig. 3), together with the fact that the oxidized form of **1** is predominant in the brain after 15 min (Fig. 2), have suggested that the accumulation at 15 min may be mainly attributable to [^{11}C]**1**. However, although pretreatment of mice with MK-801 or non-labeled **2** inhibited accumulation of the radioactivity to some extent in the regions of interest such as hippocampus, the selectivity of [^{11}C]**2** to NMDA receptors has not been clearly confirmed. As the non-cationic ligand **2** is relatively hydrophobic and has low affinity to NMDA receptors,[5] [^{11}C]**2** may be distributed to the whole brain non-selectively, and remained during the period of investigation. We anticipated that, as the cationic ligand **1** is an open channel selective ligand to NMDA receptor, [^{11}C]**1** should exhibit selective biodistribution in the NMDA-receptor rich region of the brain.[4a] However, the HPLC chromatogram of the brain homogenates at 45 min has clearly showed that the cationic ligand **1** is rapidly cleared from the brain (Fig. 2). It is reported that the channel open probability of the NMDA receptors is not very high at the normal brain activity.[12] Therefore, it may be thought that concentration of the open-state NMDA receptors in the brain is insufficient to gain selective distribution of [^{11}C]**1**.

In conclusion, this study has clearly demonstrated that **2** is useful as a prodrug for the cationic ligand **1**, an NMDA-open channel selective ligand, to enhance penetration into the brain. Although development of more potent ligand should be needed to achieve selective in vivo biodistribution to the CNS NMDA receptors, the prodrug mechanism of the ligand **2** is interesting from the view point of its pharmacological effect.

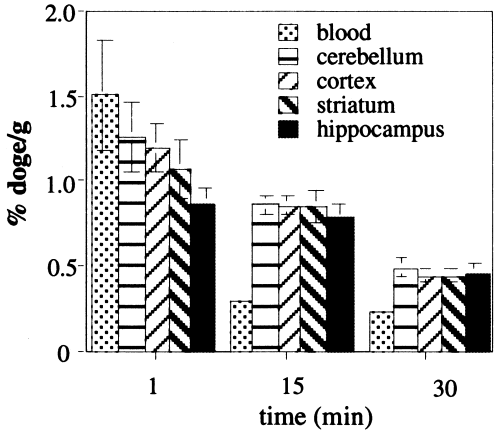


Figure 3. Biodistribution of the radioactivity in brain regions of mice following intravenous injection of [^{11}C]**2**.

Acknowledgements

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5. In vitro binding affinity to NMDA receptors was evaluated as IC_{50} values for displacement of [^{3}H]MK-801 or [^{3}H]TCP bound to rat brain homogenates. IC_{50} (**1**) = 0.019±0.002 μM for displacement of [^{3}H]TCP, IC_{50} (**2**) = 1.0±0.4 μM for displacement of [^{3}H]MK-801.
6. All compounds were used without optical resolution.
7. **5** as TFA salt: colorless oil, IR (cm^{-1}, neat): 3450, 3000; HR FABMS (*m*/*z*) calcd for $C_{23}H_{18}ONS_2$ $(M)^+$ 388.0830, found 388.0826; ^{1}H NMR (270 MHz, CD_3OD/TMS) δ (ppm) 9.16 (1H, d, *J* = 6.3 Hz), 8.34 (1H, t, *J* = 7.6 Hz), 8.01 (1H, d, *J* = 7.6 Hz), 7.74 (1H, dt, *J* = 1.3, 6.3 Hz), 7.24–7.09 (4H, m), 7.05 (1H, d, *J* = 2.6 Hz), 6.93–6.76 (4H, m), 6.60 (1H, dd, *J* = 1.2, 3.3 Hz), 6.01 (1H, s), 3.51 (1H, dd, *J* = 3.3, 14.5 Hz), 3.03 (1H, dd, *J* = 2.2, 14.5 Hz).
6: white powder, mp 152–154 °C; IR (cm^{-1}, neat): 3450, 3000; FABMS (*m*/*z*): 392 $(M+H)^+$; Anal. ($C_{23}H_{21}ONS_2$) C, H, N; ^{1}H NMR (500 MHz, CD_3OD) δ (ppm) 7.22 (1H, d, *J* = 0.9 Hz), 7.21 (1H, d, *J* = 3.2 Hz), 7.05 (1H, dd, *J* = 0.9, 3.4 Hz), 7.01 (1H, d, *J* = 7.3 Hz), 6.98 (1H, dd, *J* = 1.1, 5.3 Hz), 6.90 (1H, dd, *J* = 3.7, 5.0 Hz), 6.68 (1H, bs), 6.67 (1H, d, *J* = 2.1 Hz), 6.63 (1H, dd, *J* = 3.7, 5.0 Hz), 5.45 (2H, s), 4.20 (1H, s), 3.86 (1H, bs), 3.49 (1H, dd, *J* = 3.6, 12.8 Hz), 3.22 (1H, d, *J* = 15.6 Hz), 2.62 (1H, d, *J* = 14.6 Hz), 2.24 (1H, dd, *J* = 1.8, 12.8 Hz), 1.97 (1H, d, *J* = 10.5 Hz), 1.67 (1H, d, *J* = 16.0 Hz), 1.31 (1H t, *J* = 15.1 Hz).
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9. HPLC condition: column; Shiseido Capcell Pak 10×250 mm, solvent; 85% MeOH, flow rate; 5 mL/min, monitored at 254 nm. The retention time of **1** is 7.5 min, and that of **2** is 10.5 min.
10. The brain was homogenized in methanol, and centrifuged at 3000 rpm for 15 min in an Eppendorf microcentrifuge. The supernatant containing 75–85% of radioactivity of the whole brain was passed through a filter (DISMIC-13JP, Toyo Roshi) to remove remaining debris. HPLC[9] was then performed on a 0.6 mL sample. The unknown radioactive materials at around 5 min are observed constantly in all the homogenates during the investigation.
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