Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **XLI**, 843-858 (1998)

Synthesis and Brain Distribution of Carbon-11 Labeled Analogs of Antagonists for the NMDA Receptor Coupled PCP-Binding Site.

Terushi Haradahira[1], Sigeki Sasaki[2], Minoru Maeda[2], Kaoru Kobayashi[3], Osamu Inoue[3], Urara Tomita[4], Toru Nishikawa[4], and Kazutoshi Suzuki[1]

[1]National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555; [2]Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582. [3]Osaka University, 2-2 Yamada-Oka, Suita, Osaka 565-0871; [4]National Center of Neurology and Psychiatry, 4-1-1 Ogawa-Higashi, Kodaira, Tokyo 187-0031, JAPAN.

SUMMARY

Two phencyclidine (PCP) analogs, **3** and **4**, and one thienylcyclohexylpiperidine (TCP) analog, (±)**6**, were labeled with positron emitter carbon-11. These compounds displayed higher *in vitro* binding affinities than PCP itself for the PCP-binding site located inside the ion channel on the N-methyl-D-aspartate (NMDA) receptors. Brain distribution studies in mice showed different uptake characteristics between the PCP and TCP analogs, indicating their different *in vivo* interactions with the brain components including the PCP-binding site probably due to the different physicochemical properties of the molecules.

Key Words: NMDA receptor, phencyclidine, thienylcyclohexylpiperidine, Carbon-11, positron emission tomography

INTRODUCTION

In recent years there has been intense interest in developing radioligands for *in vivo* functional studies of the *N*-methyl-D-aspartate (NMDA) receptors using positron emission tomography (PET) or single photon emission tomography (SPECT). This interest comes from key roles of the NMDA receptors in human plastic synaptic events such as memory formation

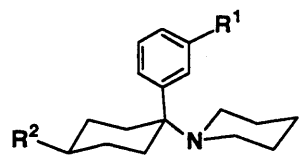
CCC 0362–4803/98/090843–16$17.50

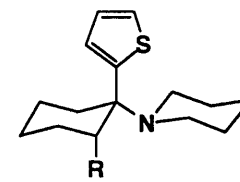
Received 23 March 1998
Revised 18 May 1998
Accepted 19 May 1998

and learning[1] as well as in neurotoxicity associated with stroke, seizure and several neurodegenerative disorders[2]. In general, a radiopharmaceutical for visualizing neurotransmitter receptors in brain is required to have at least two properties. First, it may be an agonist or antagonist that interacts with the receptor with high specificity (K_D in the nano or pico molar range). Second, it must have the ability to penetrate the blood-brain barrier (BBB) in significant quantity. The NMDA receptors possess a variety of drug binding sites in addition to L-glutamate-binding site, namely glycine-, polyamine-, zinc-, and dissociative anesthetic phencyclidine (PCP)-binding sites[3,4]. Various compounds acting as agonist or antagonist for the each drug binding site have recently been developed and extensively used *in vitro* and *in vivo* studies of the NMDA receptors[4]. Most of these compounds except for the antagonists binding to the PCP-binding site, however, have poor BBB permeability. Therefore only lipophilic PCP-binding site antagonists such as PCP, thienylcyclohexylpiperidine (TCP), and (±)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]-cycloheptan-5,10-imine (MK-801) have previously been labeled with ^{11}C, ^{18}F, and $^{123,125}I$[5,6] and have been examined for their utility as *in vivo* radioligands for PET and SPECT. None of these radioligands, however, has proved to be useful for imaging the NMDA receptors *in vivo* because of their undesirable high non-specific interactions with the brain components. Therefore, the search continues for appropriate radioligands capable of visualizing the NMDA receptors *in vivo* with PET or SPECT.

A previous study on the structure-activity relationship (SAR) of PCP[7] has shown that introduction of a methoxyl group (**3**, **4**) to the meta-position on the phenyl ring of the PCP molecule afforded considerable improvement in affinity over PCP. SAR studies of TCP which is known to exhibit about

10-fold higher *in vitro* affinity than PCP to the PCP-binding site[8] have shown that introduction of a methyl[8] or a hydroxymethyl group ((±)**5**)[9] to the C_2-position on the cyclohexyl ring is well tolerated for the activities. The *cis/trans* geometry of substituents on the cyclohexyl ring of both PCP and TCP analogs apparently affects their *in vitro* affinities[7-9]. *Cis*-substitutions at the C_2 of the TCP cyclohexyl ring, in which the piperidine ring is on the same side as the C_2-substituents such as (±)**5**, in general show relatively higher affinities than the corresponding *trans*-substitutions. Conversely *trans*-substitutions at the C_4 of the PCP cyclohexyl ring such as **4** showed higher affinities than the corresponding *cis*-substitutions such as **12**[7]. On the basis of these SAR studies, we have synthesized a racemic TCP analog,(±)**6**, having a *cis*-methoxymethyl group at the C_2 of the cyclohexyl ring and found that this compound shows a five-fold higher *in vitro* binding affinity than PCP to the PCP-binding site by competition assay for rat brain homogenates labeled with [^{3}H]TCP[5] as shown in Table 1.





PCP: R^1=R^2=H
1: R^1=OH, R^2=H
2: R^1=OH, R^2=CH_3
3: R^1=OCH_3, R^2=H
4: R^1=OCH_3, R^2=CH_3
TCP: R=H
(±)**5**: R=CH_2OH
(±)**6**: R=CH_2OCH_3

Fig. 1. Structures of PCP and TCP Analogs

Methylations of *N*-, *O*-, and *S*-nucleophiles with [^{11}C]iodomethane are of great use in introducing a short-lived ^{11}C-positron emitter ($T_{1/2}$=20.3

min) into organic molecules[10]. The compounds (3, 4, (±)6) having a methoxyl group could be easily labeled with ^{11}C without change of chemical structure by methylation of the corresponding hydroxyl precursors (1, 2, and (±)5) with [^{11}C]iodomethane. Thus we report here the radiosyntheses of ^{11}C-labeled compounds ([^{11}C]3, [^{11}C]4, and (±)[^{11}C]6) as tracer candidates for imaging the NMDA receptors with PET, as well as their different brain distributions in mice.

Table 1. Binding Affinity and Lipophilicity of PCP and TCP Analogs.

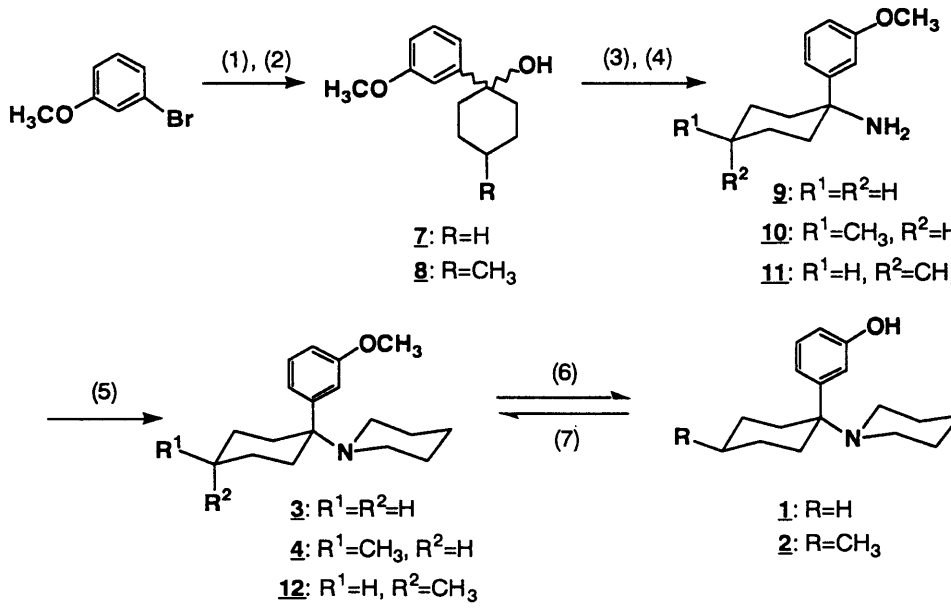
Compound	IC_{50}(nM)[a]	IC_{50}(nM)[b]	LogP[d] measured[e]	LogP[d] calculated[f]
PCP	52±7	142	1.69±0.01	3.98
3	-	-	1.92±0.01	3.72
4	-	-	2.13±0.02	4.05
12	-	-	-	-
TCP	7.94±0.03	-	2.22±0.01	3.13
(±)5	16±2	-	-	-
(±)6	-	28.6±4.7[c]	3.00±0.06	2.71

a) data from reference 9. b) values were determined as described in reference 5. c) value is the mean with S.E.M. from 4 experiments. d) logarithm of the partition coefficient between n-octanol and water. e) measured at pH=7.4 using a standard shake flask method and values reported are the mean with S.D. from 3-4 experiments. f) estimated by the fragment method of Ghose and Crippen.[21]

RESULTS AND DISCUSSION

The PCP analogs (3 and 4) were prepared by the conventional method of Geneste *et al.*[11] as shown in Scheme 1. Briefly, reaction of 3-methoxyphenylmagnesium bromide and cyclohexanone or 4-methylcyclohexanone gave tertiary alcohol (7 or 8), which was converted to tertiary

amine (**9** or **10** and **11**) *via* tertiary azide. The *cis* (**11**) and *trans* (**10**) isomers were easily separated by column chromatography on silica gel and their stereochemicals were identified by ^{1}H-NMR spectral analyses after conversion into the PCP analogs (**4** and **12**). The C_4-methyl proton signal of the *trans*-isomer (**4**) appeared up-field (δ=0.75 ppm) compared with that of the *cis*-isomer (**12**) (δ=0.95 ppm) due to the shielding effect by the phenyl ring[12]. Hydroxyl precursors (**1** and **2**) used for the radiolabeling were prepared in good yields by demethylation of **3** or **4** with hydrobromic acid in acetic acid[13]. These precursors were further purified by HPLC to prevent contamination with trace amounts of methoxy derivatives which lower the specific activity of the labeled compounds.



Scheme 1. Syntheses of PCP Analogs. (1) Mg; (2) cyclohexanone or 4-methylcyclohexanone; (3) NaN_3, CF_3CO_2H; (4) $LiAlH_4$; (5) 1,5-dibromopentane, K_2CO_3; (6) HBr/CH_3CO_2H; (7) CH_3I, NaH, DMF

Radiosynthesis was carried out in an automated synthesis apparatus for ^{11}C-labeled compounds developed by Suzuki *et al.*[14]. Reaction of **1** or **2** with [^{11}C]iodomethane in dimethylformamide in the presence of about 2eq of NaH at 50°C for 5 min gave [^{11}C]**3** or [^{11}C]**4** with 90-92% incorporation of the radioactivity, which was purified by reversed-phase HPLC and finally formulated with an isotonic saline (see Experimental for details). The total synthesis time was 22-24 min from the end of bombardment (EOB). Attempted ^{11}C-methylation of (±)**5** by the same procedure as for the syntheses of [^{11}C]**3** and [^{11}C]**4** was unsuccessful and gave (±)[^{11}C]**6** in poor yields (≤3%). This low reactivity of (±)**5** toward the ^{11}C-methylation would be due to the insufficient formation of an active *O*-nucleophile by the short treatment of (±)**5** with NaH at room temperature. This problem was overcome by heating of (±)**5** with an excess amount of dry NaH in dimethylsulfoxide before the reaction with [^{11}C]iodomethane, giving (±)[^{11}C]**6** with 41% incorporation of the radioactivity. The total synthesis time of (±)[^{11}C]**6** including HPLC purification and subsequent formulation with isotonic saline was about 24 min. The radiochemical purities and specific activities of these labeled compounds in isotonic salines were always ≥99% and ≥41GBq/μmol, respectively.

The distribution of ^{11}C in blood and in four brain regions (cerebral cortex, hippocampus, striatum, and cerebellum) was studied after intravenous injections of the ^{11}C-labeled compounds (Table 2). Initial brain uptake of the TCP analog ((±)[^{11}C]**6**) observed at 1 min after the injection was about 2-fold those of the PCP analogs ([^{11}C]**3** and [^{11}C]**4**). These three radioligands seemed to be lipophilic enough to freely enter the brain as supported by their high logP values (Table 1). Therefore, the

difference in the initial brain uptake between the PCP and TCP analogs may be due to their different initial concentrations in the blood after injection. This suggests different peripheral metabolism and elimination processes. The radioactivities of (±)[^{11}C]**6** decreased rapidly with time from all the brain regions, in parallel to the radioactivity in the blood, without showing any selective localization.

Table 2. Brain Distributions (%dose/g ± S.D.; n=3) of ^{11}C-Labeled Compounds

	Region	1 min	5 min	15 min	30 min
[^{11}C]**3**	Cerebral cortex	1.45±0.57	1.68±0.08	1.28±0.06	0.85±0.09
	Hippocampus	1.43±0.12	1.47±0.04	1.22±0.05	0.80±0.09
	Striatum	1.56±0.13	1.72±0.08	1.26±0.04	0.80±0.10
	Cerebellum	1.59±0.07	1.47±0.04	1.06±0.03	0.70±0.06
	Blood	1.54±0.22	1.16±0.11	0.88±0.03	0.71±0.08
[^{11}C]**4**	Cerebral cortex	1.38±0.14	1.20±0.17	1.15±0.08	0.97±0.10
	Hippocampus	1.09±0.04	1.05±0.16	1.09±0.07	0.88±0.11
	Striatum	1.27±0.10	1.14±0.23	1.05±0.03	0.91±0.09
	Cerebellum	1.18±0.12	1.04±0.16	1.00±0.04	0.83±0.10
	Blood	1.49±0.16	0.78±0.03	0.68±0.01	0.67±0.03
(±)[^{11}C]**6**	Cerebral cortex	2.86±0.11	1.93±0.06	0.84±0.05	0.61±0.20
	Hippocampus	2.56±0.10	1.84±0.06	0.87±0.04	0.59±0.18
	Striatum	2.75±0.17	1.89±0.08	0.91±0.10	0.60±0.17
	Cerebellum	2.88±0.13	1.81±0.07	0.81±0.04	0.59±0.16
	Blood	2.41±0.37	1.39±0.01	0.80±0.06	0.65±0.13

Contrary to the TCP analog ((±)[^{11}C]**6**), the two PCP analogs ([^{11}C]**3** and [^{11}C]**4**) showed slower clearance from the brain than that from the blood and the ^{11}C in the brain exceeded that of (±)[^{11}C]**6** at 15 min post injection. The two PCP analogs showed similar regional brain distributions. Notably they showed a slightly higher uptake in the hippocampus and striatum, regions known to be rich in NMDA receptors in rodents[15]. However the

ratio of ^{11}C in NMDA-rich regions to that in cerebellum is only ≤ 1.2, suggesting that non specific binding blurs specific binding to the NMDA receptors.

In general, a lipophilic compound having an octanol/water partition coefficient (P) that exceeds 1.0 (logP >0) is rapidly taken up by the brain from blood. The optimal lipophilicity for brain penetration has been reported to be around logP=2[16]. As shown in Table 1, the radioligands used in the present studies had measured logP values close to the optimal value, and their *in vitro* binding affinities were not significantly different from each other[7]. Therefore the observed different behaviors in the brain between the PCP and TCP analogs would indicate their different *in vivo* interactions with the brain components including the PCP-binding site. Since the PCP site is known to be located inside the ion-channels associated with the NMDA receptors[17], the bindings of the radioligands to this site would be affected by the amount and duration of the endogenous agonists such as L-glutamate and glycine both of which are essential for the channel openings. Irrespective of these agonist-dependent bindings, recent studies have suggested that the PCP-like agents may also gain access to their binding site when the channels are closed (agonist-independent bindings) as well as opened[18,19]. The NMDA ion channels are known to be closed or blocked by Mg in its resting stage, and a few of them that bind agonists have recently been reported to be actually opened[20].

Although the kinetics of the channel opening and the mode of ligand bindings (agonist-dependent or -independent) remain to be clarified to better understand the *in vivo* interactions of the radioligands with the PCP-binding site, a dominant factor determining the *in vivo* receptor-ligand interactions may be the physicochemical properties of

the radioligands such as lipophilicity and ionization *in vivo*. TCP and PCP molecules are likely to be partly ionized in the water at pH=7.4 by protonation of the basic amines[11,19]. Such ionizations of the compounds reduce their partition coefficients by making them more hydrophilic. The calculated logP value[21] of the unionized form of (±)**6** was considerably lower than those of **3** and **4** as shown in Table 1. Whereas, very interestingly, the measured logP value at pH=7.4 of (±)**6** was found to be extraordinarily high compared to those of the PCP analogs, suggesting that the TCP analog is less ionized than the PCP analogs at physiological pH, namely *in vivo*. Another possible explanation for the high measured logP value of (±)**6** could be an intramolecular hydrogen bonding between the protonated amine and the oxygen of the C_2-substituent. The importance of the hydrogen bondings in the interactions of the ligands with the pharmacophore of the ion channel has recently been reported[22,23]. Thus, it can be postulated that the rapid clearance of (±)[^{11}C]**6** from the brain is caused by reduced *in vivo* interactions not only with the PCP-binding site but also with other brain components, due to the reduced ionization and/or to the intramolecular hydrogen bonding of the molecule. In contrast, the two PCP analogs ([^{11}C]**3** and [^{11}C]**4**), which may be largely ionized *in vivo*, appeared to have some specific interactions with the PCP-binding site. However, the low ^{11}C-ratio in the regions of interest to that in the cerebellum would also indicate high non-specific interactions with brain components other than the PCP-binding site. Further investigations are required to ascertain the ionizations of the ligands affecting *in vivo* interactions with the PCP-binding site. Difference in the binding affinity among enantiomeric C_3-substituted PCP derivatives has previously been reported[24]. Therefore, the comparative

study on the brain distribution of the (+)- and (-)-isomers of *cis*-6 will also remain to be examined.

EXPERIMENTAL

General

All melting points (mp) are uncorrected. Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JNM-GX-270 spectrometer with tetramethylsilane as an internal standard. All chemical shifts (δ) are reported in parts per million (ppm) down field from the standard. Low resolution field desorption mass spectra (FD-MS) were obtained on a JEOL JMS-300 spectrometer. High resolution fast atom bombardment mass spectra (HRFAB-MS) were obtained on a JEOL NMS-SX102 spectrometer. Column chromatography was done on Merck kieselgel gel 60 F_{254} (70 - 230 mesh). Thin layer chromatography (TLC) was carried out on Merck Kieselgel 60 F_{254} plates. Radioactivity was quantified with a IGC-3R Curiemeter (Aloka). High pressure liquid chromatography (HPLC) was done using a Waters HPLC system for non-radioactive runs or a JASCO HPLC system for radioactive runs. Effluent radioactivity from the HPLC was determined using a NaI(Tl) scintillation detector system. Carbon-11 was generated by the $^{14}N(p, \alpha)^{11}C$ nuclear reaction using a CYPRIS HM-18 cyclotron (Sumitomo Heavy Industries, Ltd.). Preparation of $[^{11}C]CH_3I$ and subsequent ^{11}C-methylations were carried out automatically by using a synthetic apparatus for ^{11}C-labeled compounds developed by Suzuki et al.[14]. The free amines were converted into their hydrochloride salts by dissolving methanol saturated with hydrogen chloride and evaporated to dryness. Anhydrous DMF and DMSO were purchased from Aldrich Chemical Co. and all reagents were used as received.

N-[1-(3-methoxyphenyl)cyclohexyl]piperidine (3)

The amine intermediate (9) was prepared by the following sequence of reactions according to the method of Geneste *et al.*[11]. Reaction of 3-methoxyphenylmagnesium bromide and cyclohexanone in THF gave the tertiary alcohol (7) in 87% yield after purification by column

chromatography with hexane-ethyl acetate (9:1). Treatment of **7** with sodium azide and trifluoroacetic acid gave a tertiary azide, which was reduced to **9** with $LiAlH_4$ in 79% yield after purification by column chromatography with hexane-ethyl acetate (1:1). mp 196-198°C (HCl salt, ethyl acetate, lit.[25] 195-196°C). The free amine (**9**) was converted into **3** by a method described in the literature[26]. A solution of **9** (425 mg, 2.07 mmol) and 1,5-dibromopentane (285 µl, 2.10 mmol) in dry DMF (3.5 ml) was stirred and heated first at 60°C for 24 h, and then for a further 24 h in the presence of anhydrous K_2CO_3 (286 mg, 2.07 mmol). The reaction mixture was poured into 10% K_2CO_3 and extracted with ethyl acetate. The extract was dried over Na_2SO_4, evaporated to dryness, and the residue was chromatographed on silica gel with dichloromethane-ethyl acetate (1:2) to give pure **3** (408 mg, 72%) as a pale yellow syrup. mp 207-209°C (HCl salt, ethyl acetate, lit.[11] 206-207°C). FD-MS m/e: 273 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 7.45 (1H, t, J=8.25 Hz), 7.09 (1H, d, J=7.92 Hz), 7.06 (1H, s), 7.02 (1H, d, J=7.92 Hz), 3.88 (3H, s), 3.71 (2H, d, J=10.8 Hz), 2.83-1.06 (18H, m). Anal. Calcd for $C_{18}H_{27}NO \cdot HCl$: C, 69.77; H, 9.11; N, 4.52. Found: C, 69.41; H, 9.02; N, 4.48.

***trans and cis*-*N*-[1-(3-methoxyphenyl)-4-methylcyclohexyl] piperidine (4 and 12)**

The amine intermediates (**10** and **11**) were prepared as described in the preparation of **9**, but with 4-methylcyclohexanone used in place of cyclohexanone. The *trans* (**10**) and *cis* (**11**) isomers were obtained in 36% and 23% yields from the tertiary alcohol (**8**), respectively, after separation by column chromatography with $CHCl_3$-MeOH(19:1). Rf values were 0.16 for **10** and 0.38 for **11** on silica gel TLC plate using $CHCl_3$-MeOH-28%NH_3 (95:5:0.1) as a mobile phase. mp 211-214°C (HCl salt of **10**, acetone), 150-151°C (HCl salt of **11**, acetone). The geometrical structures of these isomers were identified by ^{1}H-NMR spectral analyses after conversion into the PCP analogs (**4** and **12**). The reaction of **10** with 1,5-dibromopentane by the same procedure described above and subsequent purification by column chromatography with $CHCl_3$-ethyl acetate (1:1) gave the *trans*-isomer (**4**) in 68% yield as a colorless syrup.

mp 223-224°C (HCl salt, ethyl acetate). ^{1}H-NMR ($CDCl_3$) δ: 7.27 (1H, t, J=8.25 Hz), 6.88 (1H, d, J=7.92 Hz), 6.84 (1H, br-s), 6.79 (1H, d, J=7.92 Hz), 3.83 (3H, s), 2.61 (2H, d, J=10.2 Hz), 2.28 (4H, br-s), 1.62-0.85 (13H, m), 0.75 (3H, d, J=6.6 Hz). HRFAB-MS (m/e) calcd for $C_{19}H_{30}NO$ (M^++H): 288.2327. Found: 288.2321.

The *cis*-isomer (**12**) was similarly obtained in 57% yield as a white solid after treatment of **11** with 1,5-dibromopentane and subsequent purification by column chromatography with petroleum ether-ether (10:1). mp 44-45°C (EtOH). ^{1}H-NMR ($CDCl_3$) δ: 7.24 (1H, t, J=8.24 Hz), 6.89 (1H, d, J=7.92 Hz), 6.84 (1H, br-s), 6.77 (1H, d, J=7.92 Hz), 3.81 (3H, s), 2.53 (2H, d, J=11.5 Hz), 2.24 (4H, br-s), 1.55-1.27 (13H, m), 0.95 (3H, d, J=5.28 Hz). HRFAB-MS (m/e) calcd for $C_{19}H_{29}NO$ (M^+): 287.2249. Found: 287.2250.

N-[1-(3-hydroxyphenyl)cyclohexyl]piperidine (1)

A mixture of **3** (20.6 mg, 0.075 mmol), 47% hydrobromic acid (0.4 ml) and acetic acid (0.4 ml) was stirred and heated at 120°C for 3 h[13]. After removal of the solvents *in vaccuo*, the residue was dissolved in water. The water was made basic to litmus with 28% NH_3 and extracted with chloroform. The organic layer was dried over Na_2SO_4 and evaporated to dryness to give **1** (18.1 mg, 93%), which was recrystallized from MeOH. mp 201-202°C. FD-MS m/e: 259 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 9.14 (1H, s), 7.09 (1H, t, J=7.59 Hz), 6.70 (1H, d, J=7.59 Hz), 6.68 (1H, s), 6.60 (1H, d, J=7.1 Hz), 2.19-1.22 (20H, m). To prevent contamination with a trace amount of **3**, the obtained **1** was further purified by HPLC (column: MegaPak SIL C18, JASCO, 10 x 250 mm) using MeOH/H_2O/triethylamine (80/20/0.05) as the mobile phase.

N-[1-(3-hydroxyphenyl)-4-methylcyclohexyl]piperidine (2)

The same treatment of **4** (201 mg, 0.67 mmol) with a mixture of 47% hydrobromic acid (3.7 ml) and acetic acid (3.7 ml) and subsequent work up as described for the synthesis of **1** gave **2** (179 mg, 94%), which was recrystallized from EtOH. mp 212-215°C (dec.). FD-MS m/e: 273 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 8.96 (1H, s), 7.09 (1H, t, J=8.1 Hz), 6.70 (2H, br-s), 6.60 (1H, d, J=7.8 Hz), 2.42 (2H, d, J=13.2 Hz), 2.22 (4H, br-s), 1.59-0.80

(13H, m), 0.74 (3H, d, J=5.9 Hz). To prevent contamination with a trace amount of **4**, the obtained **2** was further purified by HPLC (column: MegaPak SIL C18, JASCO, 10 x 250 mm) using MeOH/H_2O/triethylamine (80/20/0.05) as the mobile phase.

(±)-*N*-[1-thienyl-2-methoxymethylcyclohexyl]piperidine ((±)6)

A solution of (±)**5** (10.2 mg, 3.65×10^{-5} mol) and NaH (60% in oil, 7 mg, 17.5×10^{-5} mol) in dry DMSO (1 ml) was stirred at 50°C for 30 min under an argon atmosphere. To this solution was added iodomethane (12 µl, 19.3 $\times 10^{-5}$ mol) and the mixture was stirred at 30°C for 3 h, quenched with aqueous $NaHCO_3$, and extracted with ether. The extract was dried over Na_2SO_4 and evaporated to dryness. The residue was chromatographed on silica gel with n-hexane/ethyl acetate (4/1) to give pure (±)**6** (9.5 mg, 88.7 %) as a colorless oil. FAB-MS m/e: 293 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 7.19 (1H, dd, J=3.8, 1.0 Hz), 7.01 (1H, dd, J=3.8, 1.65 Hz), 6.80 (1H, dd, J=2.64, 1.0 Hz), 3.79 (1H, dd, J=9.9 , 3.6 Hz), 3.57 (1H, t, J=9.9 Hz), 3.40 (3H, s), 2.7-2.8 (1H, m), 2.0-2.4 (4H, m), 1.8-1.9 (1H, m), 1.2-1.7 (13H, m).

Radiosyntheses of [^{11}C]3 and [^{11}C]4

[^{11}C]Iodomethane prepared from [^{11}C]CO_2 by conventional means was introduced into a reaction vessel containing **1** or **2** (1.0 mg) and NaH (5 µl, 0.5 g/20 ml DMF) in dry DMF (250 µl) at -50°C. The temperature of the reaction vessel was raised to 50°C and the mixture was stirred for 5 min. After addition of a HPLC mobile phase (500 µl), the reaction mixture was transferred onto a reversed-phase HPLC column (MegaPak SIL C18, JASCO, 10 x 250 mm) and eluted with MeOH/H_2O/triethylamine (80/20/0.05) at a flow rate of 9 min/min. The eluate with a radioactive peak at t_R=7.6 min (for [^{11}C]**3**) or at t_R=8.1 min (for [^{11}C]**4**) was collected in a flask and the solvents were evaporated under reduced pressure. After adding isotonic saline (5-10 ml) to the flask, the radioactivity in the flask was recovered into a sterilized vial through a 0.22 µm Millipore filter to give radiochemically pure (≥ 99%) [^{11}C]**3** or [^{11}C]**4** (0.37 - 1.33 GBq) in a synthesis time of 22-24 min from EOB. The specific activities

of the ^{11}C-labeled compounds were estimated by UV spectroscopy(285 nm) to be 41GBq/μmol for [^{11}C]**3** and 63 GBq/μmol for [^{11}C]**4** at EOS.

Radiosynthesis of (±)[^{11}C]6

Dry NaH powder was obtained by several washings of a 60% NaH dispersed in mineral oil with dry hexane and subsequent drying under reduced pressure for 2h. The precursor (±)(**5**) (2.2 mg) was treated with the dry NaH (1-2 mg) at 50°C for 30 min in dry DMSO (500 μl). To this solution was added [^{11}C]iodomethane at 20°C and the mixture was heated at 50°C for 5 min. The radioactive product (t_R =7.4 min) was purified by HPLC (MegaPaK SIL C18; MeOH/H_2O/triethylamine=80/20/0.05; 4 min/min; UV at 254 nm) and formulated in an isotonic saline as described above. By this procedure radiochemically pure (≥ 99%) (±)[^{11}C]**6** (200 - 590 MBq) was obtained in a synthesis time of about 24 min from EOB.

Brain Distribution

The ^{11}C-labeled ligand was injected (ca 7.4 MBq, 37 MBq/ml) into ddY mouse (8 - 9 W.O.) through the tail vein. Animals were killed under ether anesthesia at various time points. The brain was rapidly removed, dissected into cerebellum, cerebral cortex, striatum, and hippocampus, and weighed. The radioactivity in each sample was measured in a Packard autogamma scintillation counter and corrected for decay. The results are expressed as the percent administered dose per gram of tissue (% dose/g).

LogP Determination

The logP values were measured using a standard shake flask method as follows. The sample (3- 10 mg, free base) was well shaken with a mixture of 1-octanol (2.5 ml) and phosphate buffer (2.5 ml, pH=7.4) for 30 min at 23±1°C. After centrifugation (3000 rpm × 10 min) of the mixture, the two layers were separated and the concentration of partitioned substance in each layer was quantified by HPLC (CAPCELL PAK C_{18}UG80, 4.6 mmϕ×250 mm, SHISEIDO; MeOH/H_2O/triethylamine=90/10/0.05). The mean logP values were determined after the three separate determinations and are listed in Table 1.

Acknowledgements - We thank Mr Nakano T. and Takai N.for their support of the animal experiments. We thank the cyclotron crew of the National Institute of Radiological Sciences for their help in the radioisotope production. This work has been supported in part by CREST of Japan Science and Technology Corporation.

References

1) Collingridge G.L. -*Trends Pharmacol. Sci.* **6**: 407 (1985); Morris R.G.M., Anderson E., Lynch G.S., and Baudry M. -*Nature* **319**: 774 (1986)

2) Beal M.F. -*Curr. Op. Neurobiol.* **2**: 657 (1992); Choi D.W. -*Science* **258**: 241 (1992); Dingledine R., McBain C.J., and McNamara J.O. -*Trends Pharmac. Sci.* **11**: 334 (1990); Meldrum B. and Garthwaite J. -*Trends Pharmac. Sci.* **11**: 379 (1990).

3) MacBain C.J. and Mayer M.L. -*Physiol. Rev.* **74**: 723 (1994).

4) Ishimaru M.J. and Toru M. -*Pharmacol. Pathphysiol.* **7**: 47 (1997); Leeson P.D. and Iversen L.L. -*J. Med. Chem.* **37**: 4053 (1994).

5) Shibayama Y., Sasaki S., Tomita U., Nishikawa T., and Maeda M. -*J. Label. Compods. Radiopharm.* **38**: 77 (1995), and references cited therein.

6) Ouyang X., Mukherjee J., and Yang Z.-Y. -*Nucl.Med. Biol.* **23**: 315 (1996) and references cited therein.

7) Chaudieu I., Vignon J., Chicheportiche M., Kamenka J.-M., Trouiller G., and Chicheportiche R. -*Pharmacol. Biochem. Behav.* **32**: 699 (1989).

8) Vignon J., Chicheportiche R., Chicheportiche M., Kamenka J.-M., Geneste P., and Lazdunski M. -*Brain Res.* **280**: 194 (1983).

9) Tsukiyama S., Hashimoto A., Katayama S., Nishikawa T., Tobe A., and Maeda M. -*Chem. Pharm Bull.* **39**: 1581 (1991).

10) Stöklin G. and Pike V. W. -*Radiopharmaceuticals for positron emission tomography. Methodological aspects.* Kluwer Academic Publishers, Dordrecht, Netherlands.

11) Geneste P., Kamenka J.M., Ung M.S.N., Herrmann P., Goudal R., and Trouiller G. -*Eur. J. Med. Chem. Chimi. Terap.* **14**: 301 (1979).

12) Geneste P., Herrmann P., Kamenka J.M., and Pons A. -*Bull. Soc. Chim. France.* **1975**: 1619.

13) Kawasaki I., Matsuda K., and Kaneko T. -*Bull. Chem. Soc. Japan* **44**: 1986 (1971).

14) Suzuki K., Inoue O., Hashimoto K., Yamasaki T., Kuchiki M., and Tamate K. -*Appl. Radiat. Isot.* **36**: 971 (1985).

15) Ferrarese C., Guidotti A., Costa E., Miletich R.S., Rice K.C., deCosta B.R., Fulham M.J., and DiChiro G. -*Neuropharmacol.* **30**: 899 (1991); Maragos W.F., Penney J.B., and Young A.B. -*J.Neurosci.* **8**: 493 (1988).

16) Dischino D.D., Welch M.J., Kilbourn M.R., and Raichle M.E. -*J. Nucl. Med.* **24**: 1030 (1983); Glave W.R. and Hansch C. -*J. Pharm. Sci.* **61**: 589 (1972); Greig N.H. -*Cancer Treat. Rev.* **14**: 1 (1987).

17) Honore T., Drejer J., Nielsen E.O., Watkins J.C., Olverman H. J., and Nielsen M. -*Eur. J. Pharmacol.* **172**: 239 (1989); Reynolds I.J. and Miller R.J. -*Mol. Pharmacol.* **33**: 581 (1988)

18) Davis S.N., Martin D., Millar J.D., Aram J.A., Church J., and Lodge D. -*Eur. J. Pharmacol.* **145**: 141 (1988); Javitt D.C. and Zukin S.R. -*Mol. Pharmacol.* **35**: 387 (1989).

19) Mallamo J.P., Earley W.G., Kumar V., Subramanyam C., Dority,Jr. J.A., Miller M.S., DeHaven-Hudkins D.L., Ault B., Herrmann,Jr. J.L., Dung J.-S., McMullen, L.A. Jaeger E., Kullnig R., and Magee L.J. -*J. Med. Chem.* **37**: 4438 (1994)

20) Jahr C.E. -*Science* **255**: 470 (1992); Rosenmund C., Feltz A., and Westbrook G.L. -*J. Neurosci.* **15**: 2788 (1995).

21) Ghose A.K. and Crippen G.M. -*J. Comp. Chem.* **7**: 565 (1986).

22) Leeson P.D., Carling R.W., James K., Smith J.D., Moore K.W., Wong E.H.F., and Baker R. -*J. Med. Chem.* **33**: 1296 (1990).

23) Kroemer R.T., Koutsilieri E., Hecht P., Liedl K.R., Riederer P., and Kornhuber J. -*J. Med. Chem.* **41**: 393 (1998).

24) Thurukauf A., Hillery P., Mattson M.V., Jacobson A.E., and Rice K.C. -*J. Med. Chem.* **31**: 1625 (1988).

25) Thurkauf A., deCosta B., Yamaguchi S., Mattson M.V., Jacobson A.E., Rice K.C., and Rogawski M.A. -*J. Med. Chem.* **33**: 1452 (1990)

26) Orita K., Sasaki S., Maeda M., Hashimoto A., Nishikawa T., Yugami T., and Umezu K. -*Nucl. Med. Biol.* **20**: 865 (1993).

