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Synthesis and in vivo evaluation of a new PET radioligand for studying sigma-2 receptors

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Abstract—The cyclohexyl piperazine **1** (1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-1-yl)-propyl]-piperazine) has been shown to be a potent and selective sigma-2 receptor ligand. In the present study, we prepared [^{11}C]**1** by *O*-alkylation of the phenolic precursor **2** with [^{11}C]CH_3I. [^{11}C]**1** was obtained in a 29% non-decay corrected yield and specific activity of 9299 mCi/μmol calculated at end-of-synthesis. The biodistribution of [^{11}C]**1** in mouse brain demonstrated rapid and homogenous concentration in all brain structures, which included the cortex, thalamus, cerebellum and striatum. Co-administration of unlabelled **1** (1 mg/kg) or the sigma-2 selective ligand SM-21 (1 mg/kg) failed to show any significant inhibition of [^{11}C]**1** uptake in the mouse brain. The evaluation of this radioligand in vivo in the mouse clearly indicates that it does not possess the required properties for studying sigma-2 receptors in the brain using PET.

1. Introduction

Sigma receptors were originally thought to be a type of opioid receptor to account for the actions of racemic benzomorphans and then identified with the phencyclidine site on the NMDA receptor channel. However, subsequent studies revealed that sigma receptors are a distinct class of receptors that are expressed in peripheral organs such as the liver, kidneys, endocrine glands and the central nervous system (CNS). There are at least two identified subtypes, namely sigma-1 and sigma-2 sites.[1] The sigma-1 receptor has been identified to be a mammalian homologue of the yeast sterol isomerase, while the molecular identity of the sigma-2 receptor has not yet been fully resolved.[2,3]

Since the discovery of sigma receptors, research has been ongoing in an attempt to understand the functional roles of these sites. Initial interest in sigma receptors was largely motivated by the observation that the sigma site was a high affinity binding site for psychoactive drugs including many of the atypical antipsychotic drugs.[4,5] Ligands, which bind with high affinity at sigma receptors have been shown to modulate and interfere with several neurotransmitters and have potent activities in animal models suggestive of antipsychotic, cognitive enhancing, neuroprotective and antidepressant activities.[6–8] Several trials performed with sigma-1 antagonists have shown that these ligands are effective against negative symptoms of schizophrenia and did not induce extrapyramidal side effects, while sigma-2 receptors have been implicated in motor-side effects of antipsychotic drugs suggesting that sigma-2 antagonists may be useful in the treatment of psychoses.[9,10] Therefore, tomographic imaging of sigma receptors might be useful in providing a better understanding of the physiological role of sigma receptors in these disorders.

Although several radioligands have been developed for the sigma-1 receptor, there are currently no suitable sigma-2 radioligands for use with positron-emission-tomography (PET),[11,12] while some new radioligands are currently under evaluation.[13] Recently, a series of *N*-aryl and *N*-alkylpiperazines derivatives have been reported with high affinity for sigma-2 receptors.[14,15] The

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most promising candidate from this series, 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-1yl)-propyl]-piperazine, **1**, displayed higher affinity for sigma-2 receptors (K_i = 0.34 nM using rat liver membranes) compared to sigma-1 receptors (K_i = 13.6 nM using whole guinea-pig brain) and lower affinity for 5-HT_{1A} (K_i = 258 nM) and dopamine D_2 receptors (K_i = 604 nM). Based on these in vitro results and the fact that this compound contains a methoxy moiety make it a suitable candidate for radiolabelling using carbon-11. In this paper, we report the synthesis, radiosynthesis and preliminary evaluation of [^{11}C]**1** in the mouse brain.

2. Results and discussion

2.1. Chemistry

The intermediates and target compound **1** (1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-1-yl)-propyl]piperazine) were synthesised according to the literature methods.[14,16–18] The phenolic precursor **2** for radiolabelling was synthesised as outlined in Scheme 1. Demethylation of the methoxy moiety of **1** was carried out using HBr and the phase transfer catalyst tetra-*n*-butylphosphonium bromide.[19] The resulting phenolic compound **2** was obtained as a semi-solid in 70% yield and was used as the precursor in subsequent radiolabelling reactions.

2.2. Radiochemistry

[^{11}C]**1** was prepared by *O*-alkylation of the phenolic precursor **1** with [^{11}C]CH_3I. The reaction was performed in DMF using tetrabutylammonium hydroxide (TBAH) and heated at 80 °C for 2 min. Purification was carried out by C-18 semi-prep HPLC followed by evaporation and formulation. This resulted in an average radiochemical yield of 29 ± 5% (n = 6) non-decay corrected and specific activity of 9299 Ci/mmol calculated at end-of-synthesis (Scheme 2).

2.3. Rodent biodistribution studies

Following intravenous (iv) injection of [^{11}C]**1** into mice, the time course of radioactivity in several brain regions was examined (Fig. 1). The in vivo biodistribution of [^{11}C]**1** in mice showed a high uptake in the posterior cortex, temporal cortex and cerebellum (2.3% ID/g) as early as 5 min. High uptake was also observed in the frontal cortex and thalamus (2.2% ID/g) as well as in the striatum (2.0% ID/g). At 15 min, the uptake in the posterior cortex, temporal cortex and cerebellum was slightly increased (2.4% ID/g) with the same trend also observed in the other brain regions. The radioligand uptake continued to increase at 30 min and peaked at 60 min

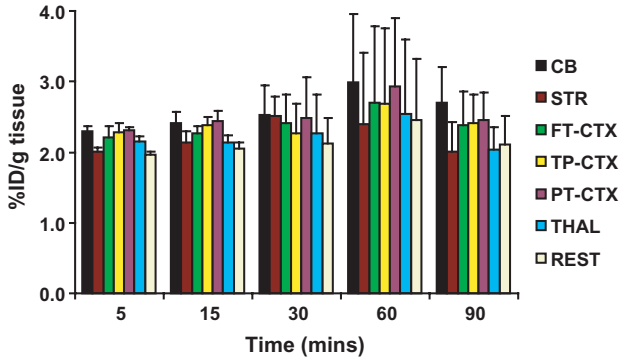
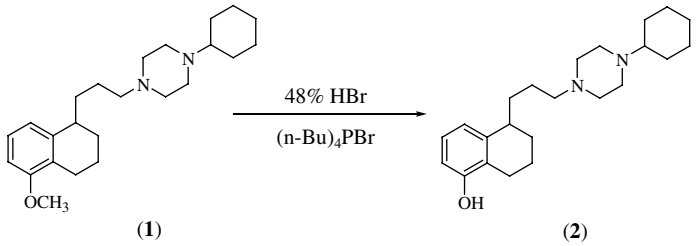
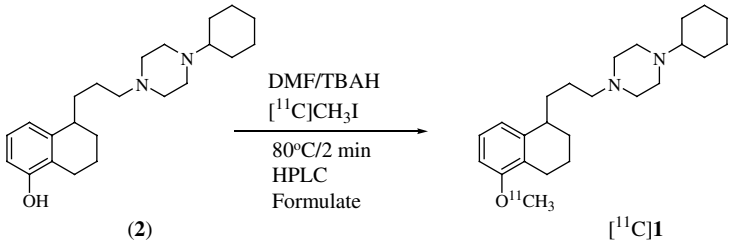


Figure 1. Time course of [^{11}C]**1** in the mouse brain. CB = cerebellum, STR = striatum, FT-CTX = frontal cortex, TP-CTX = temporal cortex, PT-CTX = posterior cortex, THAL = thalamus, REST = rest of brain. Data are means ± SEM; n = 3.



Scheme 1. Synthesis of 5-[3-(4-cyclohexyl-piperazin-1-yl)-propyl]-5,6,7,8-tetrahydro-naphthalen-1-ol (**2**) for radiolabelling reactions. The methods of Perrone et al.[16,17] and Berardi et al.[18] were used to synthesise **1**.



Scheme 2. Radiosynthesis of [^{11}C]**1**.

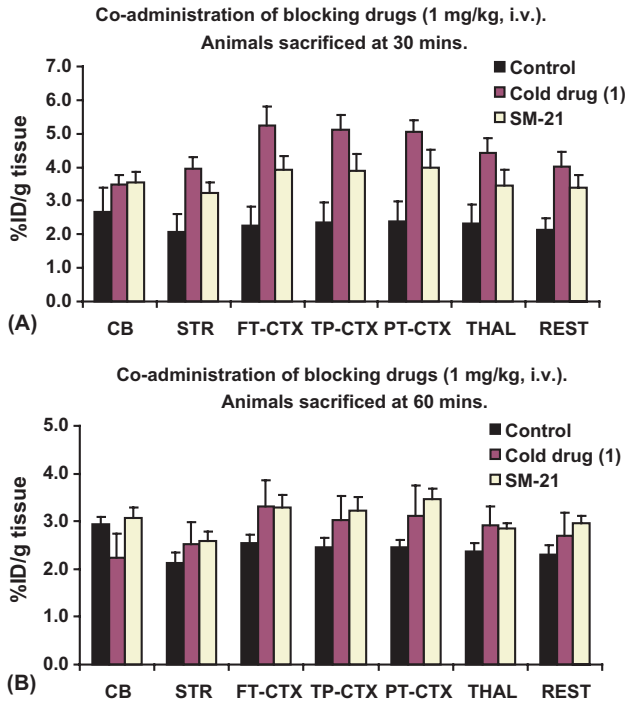


Figure 2. Effect of unlabelled **1** (1 mg/kg) and SM-21 (1 mg/kg) on [^{11}C]**1** binding at 30 min (A) and 60 min (B) post-injection. CB = cerebellum, STR = striatum, FT-CTX = frontal cortex, TP-CTX = temporal cortex, PT-CTX = posterior cortex, THAL = thalamus, REST = rest of brain. Data are means ± SEM; $n = 3$.

post-injection (p.i.) with the cerebellum and posterior cortex 3.0% ID/g, frontal and temporal cortex 2.7% ID/g, thalamus 2.5% ID/g and striatum 2.4% ID/g. At 90 min p.i. the uptake resembled values obtained at 30 min.

To determine the degree of specific binding and the extent of selective sigma-2 binding, blocking studies were performed (Fig. 2). Co-administration of unlabelled **1** at 1 mg/kg (iv) had no inhibitory effect on [^{11}C]**1** uptake in any brain region at 30 or 60 min. On the contrary, an increase in [^{11}C]**1** uptake was observed in all brain regions. This increase was greater at 30 min than 60 min. This may be an effect of unlabelled **1** displacing [^{11}C]**1** from peripheral pools resulting in a higher brain uptake. Co-administration of the selective sigma-2 ligand SM-21 at 1 mg/kg (iv) also had no inhibitory effect on [^{11}C]**1** uptake in any brain region at 30 or 60 min. Again an increase in [^{11}C]**1** uptake was observed at both time points but was lower than that observed with unlabelled **1**. These in vivo results clearly demonstrate that the observed brain uptake following [^{11}C]**1** administration represents non-specific binding.

3. Conclusion

The aim of this preliminary study was to prepare 1-cyclohexyl-4-[3-(5-methoxy-1,2,3,4-tetrahydro-naphthalen-1yl)-propyl]-piperazine, **1**, in radiolabelled form using carbon-11 and determine the ability of this radioligand for binding to sigma-2 receptors in vivo in the mouse brain. [^{11}C]**1** was prepared at high specific radioactivity by *O*-alkylation of the phenolic precursor **2** with [^{11}C]CH_3I. The radiosyntheses produced radiochemically pure [^{11}C]**1** in good radiochemical yield. The evaluation of this radioligand in vivo in the mouse clearly indicates that it does not possess the required properties for studying sigma-2 receptors in the brain using PET.

4. Materials and methods

4.1. General

All reagents and solvents used were obtained from commercially available sources and used with no further purification. All synthesised compounds were examined by thin-layer chromatography on silica and alumina. Unless specified otherwise, ^{1}H and ^{13}C NMR spectra were recorded as solutions in $CDCl_3$ on a Varian 300 MHz instrument (300 MHz for ^{1}H and 75.46 MHz for ^{13}C). Mass spectra were recorded on a Finnigan/MAT TSQ46 using chemical ionisation (C.I.) by the Department of Pharmacy, the University of Sydney. All animal procedures were carried out in full compliance with institutional guidelines relating to the conduct of animal experiments. [^{11}C]Methyl iodide was produced using a GE PETtrace cyclotron and the GE Microlab. High performance liquid chromatography analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line fixed wavelength (254 nm) UV detector and a single sodium iodide crystal flow radioactivity detector. HPLC chromatograms were recorded by a Ranin Dynamax dual channel control/interface module connected to a MacIntosh computer with the appropriate program software (Dynamax version 1.4).

4.2. Chemistry

4.2.1. 5-[3-(4-Cyclohexyl-piperazin-1-yl)-propyl]-5,6,7,8-tetrahydro-naphthalen-1-ol (2). A mixture of **1** (0.45 g, 1.22 mmol), tetra-*n*-butylphosphonium bromide (0.04 g, 0.12 mmol) and 48% HBr (6 mL) was heated at 100 °C for 6 h. The reaction mixture was evaporated to reduce the volume, then basified with saturated $NaHCO_3$ to pH 8–9 and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and removal of solvent gave 0.4 g of a brown oil. Purification by column chromatography eluted with 0–2% $CH_3OH/CHCl_3$ yielded **2** as a light brown semi-solid (0.3 g, 70% yield). ^{1}H NMR ($CDCl_3$): δ 1.0–1.4 (m, 5H, cyclohexyl); δ 1.5–2.0 (m, cyclohexyl NC*H*, NCH_2 and benzylic, 13H); δ 2.2–2.9 (m, 14H, piperizine, NC*H*, NCH_2 and benzylic); δ 6.55 (d, 1H, $J = 7.80$ Hz, ArCH); δ 6.73 (d, 1H, $J = 7.80$ Hz, ArCH); δ 6.9 (t, 1H, $J = 7.80$ Hz, ArCH). ^{13}C NMR ($CDCl_3$): δ 20.0, 24.3, 25.7, 27.1, 27.4, 28.0, 30.0, 35.8, 38.8, 49.8, 54.6, 60.1, 64.8, 113.0, 121.5, 125.3, 126.9, 144.1, 155.1; MS (M+1), 357 (100), 259 (75).

4.3. Radiochemistry

4.3.1. Radiosynthesis of [^{11}C]1. The phenolic compound **2** (0.5 mg) was dissolved in dimethylformamide (200 μL)

and transferred to a small septum-sealed vessel followed by the addition of tetrabutyl ammonium hydroxide (1.5 μL, 1.0 M in CH_3OH). The vessel was cooled (−78 °C) and [^{11}C]methyl iodide, produced in a General Electric Microlab from [^{11}C]CO_2,[20] was transferred into the vessel by a stream of helium carrier gas. When the radioactivity in the solution reached a plateau, the stream of helium was stopped and the vessel was submerged in an 80 °C water bath. After 2 min, 200 μL of HPLC solvent, consisting of 50:50 acetonitrile–0.1 M NH_4HCO_2, was added to the reaction solution. The mixture was injected onto a NovaPak column (6 μm 7.8 × 200 mm) and eluted at a flow rate of 8 mL/min. The effluent from the column was monitored with a UV detector and an in-line radioactivity detector. The radioactivity peak corresponding to [^{11}C]**1** (t_R = 5.9 min) was collected in a rotary evaporator and the solvent evaporated to dryness under reduced pressure. The residue was dissolved in sterile, normal saline (7 mL) and filtered through a sterile, Millipore GS 0.22 μm filter, into a sterile pyrogen-free evacuated vial. Sterile aqueous sodium bicarbonate (3 mL, 8.4%) was then added and the radioactivity was measured.

For determination of specific radioactivity and radiochemical purity, an aliquot of the final solution of known volume and radioactivity was injected onto an analytical reverse-phase HPLC column (NovaPak 4 μm 3.8 × 150 mm). A mobile phase of 50:50 acetonitrile–0.1 M NH_4HCO_2, with a flow rate of 4 mL/min, was used to elute the radioligand (t_R = 1.4 min). The area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured by an automated integrating recorder (Hewlett Packard 3390A) and compared to a standard curve relating mass to UV absorbance.

4.4. Rodent biodistribution studies

Male CD1 mice (Charles River, Wilmington, MA) weighing between 25 and 30 g were used in all experiments. Each mouse received an intravenous (iv) injection of 200 μCi of [^{11}C]**1** in a volume of 0.2 mL of 70:30 normal saline–sodium bicarbonate. For kinetic studies, groups of three animals were sacrificed by cervical dislocation at 5, 15, 30, 60 and 90 min post-injection. The whole brain was removed quickly, placed on ice and samples of cerebellum, striatum, frontal cortex, temporal cortex, posterior cortex, thalamus and the rest of the brain were dissected and weighed. The tissue radioactivity was measured with an automated gamma counter and the percent injected dose (% ID/g) of wet tissue was calculated by comparison with samples of standard dilutions of the initial dose. All radioactivity measurements were corrected for decay. The specificity of [^{11}C]**1** binding to sigma receptors in vivo was investigated by co-administering 1 mg/kg of unlabelled **1** and the sigma-2 selective ligand SM-21. The animals were sacrificed at 30 and 60 min after injection of the radioligand and the tissues were handled as described above.

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