NeuroImage 45 (2009) 687–693

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Validation of 4-[^{18}F]-ADAM as a SERT imaging agent using micro-PET and autoradiography

Kuo-Hsing Ma [a,*], Wen-Sheng Huang [b], Yu-Yeh Kuo [a], Chi-Jiun Peng [b], Nien-Hsien Liou [a], Ren-Shyan Liu [c], Jeng-Jong Hwang [d], Jiang-Chuan Liu [a], Haw-Jan Chen [e], Chyng-Yann Shiue [b]

[a] Department of Biology and Anatomy, National Defense Medical Center, Taipei, No.161, Sec. 6, Min-Chuan E. Road, Neihu 114, Taipei, Taiwan
[b] Department of Nuclear Medicine, Tri-Service General Hospital, Taipei, Taiwan
[c] Department of Nuclear Medicine, National Yang-Ming University, Taipei, Taiwan
[d] Department of Biomedical Imaging and Radiological Science, National Yang-Ming University, Taipei, Taiwan
[e] Institute of Nuclear Energy Research, Taoyaun, Taiwan

ARTICLE INFO

Article history:
Received 20 August 2008
Revised 4 December 2008
Accepted 23 December 2008
Available online 13 January 2009

Keywords:
5,7-Dihydroxytryptamine
p-Chloroamphetamine
Paroxetine
Positron emission tomography
Autoradiography
Serotonin transporters
4-[^{18}F]-ADAM

ABSTRACT

Serotonin transporters (SERTs) have been implicated in various neuropsychiatric disorders. We aim to validate 4-[^{18}F]-ADAM (*N*,*N*-dimethyl-2-(2-amino-4-[^{18}F]fluorophenylthio)benzylamine) as a SERT imaging agent in rats using micro-positron emission tomography (micro-PET) and autoradiography. Sixty to ninety min after injecting 4-[^{18}F]-ADAM, specific uptake ratios (SURs) were determined by micro-PET measurements in various brain regions of normal control rats. For $n=3$, the SUR in the midbrain was 4.94±0.16, for the hypothalamus it was 4.39±0.031 and for the caudate it was 4.18±0.53. The retention of 4-[^{18}F]-ADAM in the hypothalamus and midbrain regions increased rapidly between 5 to 10 min after injection and declined thereafter. The SURs determined by autoradiography were: 9.31±1.41 for the midbrain, 7.15±1.45 for the hypothalamus and 5.22±1.14 for the caudate putamen. Both micro-PET and autoradiography studies revealed a dose-dependent progressive inhibition of radioligand uptake in the frontal cortex, caudate putamen and hypothalamus in rats treated with 0.01 to 0.25 mg/kg paroxetine. A decrease in 4-[^{18}F]-ADAM uptake of approximately 84% was observed in the midbrain of rats pretreated with 0.25 mg/kg paroxetine as compared to controls (4.94±0.16 versus 0.80±0.17, $n=3$). Both 5,7-dihydroxytryptamine and *p*-chloroamphetamine-treated rats showed pronounced reduction in 4-[^{18}F]-ADAM binding when compared to normal controls. Rats pretreated with *p*-chloroamphetamine exhibited significant inhibition of 4-[^{18}F]-ADAM uptake in brain regions rich in SERT over a period of four weeks. Thus, 4-[^{18}F]-ADAM is a SERT-specific radioligand that may be useful for evaluating neuropsychiatric conditions involving serotonergic dysfunction.

Introduction

The presynaptic serotonin transporter (SERT) is an oligomeric protein which regulates serotonin (5-hydroxytryptamine, 5-HT) concentration by controlling reuptake of serotonin in the synaptic cleft (Snyder and Ferris, 2000). Serotonin transporters are localized primarily on axons concentrated at varicosities and terminal buttons in the raphe nuclei and thalamus (Zhou et al., 1998). Although alterations to the SERT primary structure have not been directly associated with clinical disorders, gene polymorphisms affecting serotonin uptake have been correlated with various neuropsychiatric diseases, including Parkinson's disease, depression, suicide, schizophrenia, eating disorders and drug addiction (Meltzer et al., 1998; Mann et al., 2000). Multiple factors, including plasma membrane concentration (Scanlon et al., 2001), interleukin (Mössner et al., 2001), glucocorticoids (Glatz et al., 2003), and growth factors (Mössner et al., 2000), also play a role in the uptake of serotonin, a process on which pharmacological and diagnostic assays are based.

Non-invasive imaging modalities, such as positron emission tomography (PET), magnetic resonance imaging (MRI), and single-photon emission computed tomography (SPECT) have recently gained importance as tools for demonstrating proof of concept in drug development studies which focus on brain monoamines (Hartvig et al., 2002). PET, for example, uses distinctly labelled compounds to visualize SERT in brain tissue (Brust et al., 2003; Ginovart et al., 2003; Huang et al., 2004; Jarkas et al., 2005; Lundquist et al., 2007).

Of the initial radioligands developed for SERT imaging, [^{11}C]-labelled citalopram, fluoxetine and associated derivatives were unsuccessful due to their unfavourable specific-to-nonspecific binding ratios and the short half-life of ^{11}C (Scheffel et al., 1994). In the 1990's, [^{11}C](+)McN-5652 was used as a PET tracer to assess serotonin transporter density (Suehiro et al., 1993; Szabo et al., 1995). However, the use of this tracer for SERT imaging is limited because the brain uptake kinetic is not optimal. Separation of specific from non-specific binding does not occur until later in a study, thus at least 120 min of

* Corresponding author. Fax: +886 2 87923159.
E-mail address: kuohsing91@yahoo.com.tw (K.-H. Ma).

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doi:10.1016/j.neuroimage.2008.12.060

data acquisition in humans is necessary to yield time-independent measures of SERT binding potential (BP) in regions with high SERT density, such as the midbrain. In addition, the level of non-specific binding is relatively high, thus precluding the reliable quantification of SERT in regions of relatively low SERT density such as the limbic system. Finally, the metabolism of [^{11}C](+)-McN 5652 in peripheral plasma is rapid and the plasma free fraction is low, making it impossible to control for this variable in clinical studies.

Recently, *N*,*N*-dimethyl-2-(2-amino-4-chlorophenylthio)benzyl alcohol (403U76) was reported to have high selectivity and potent binding affinity for SERT (K_i=0.013 nM) over NET (K_i=699 nM) or DAT (K_i=840 nM) (Ferris et al., 1995). Its analog, [^{11}C]*N*,*N*-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine ([^{11}C]DASB), was subsequently synthesized (Wilson et al., 2000) and shown to be a potential radioligand for PET studies of SERT in humans (Houle et al., 2000). However, compared to ^{11}C, ^{18}F has some advantages (Shiue et al., 2003b). ^{18}F has lower positron energy than that of ^{11}C (0.635 vs 0.96 MeV) and thus has higher spatial resolution. In addition, due to the longer half-life of ^{18}F a more optimized scanning protocol is possible. Finally, ^{18}F is convenient for radiosynthesis and the radioligands can be transported off site, if a cyclotron is not available.

The ^{8}F analogs such as ^{8}F-ACF (2-[2-amino-4-chloro-5-[^{18}F]fluorophenyl]thio)-*N*,*N*-dimethylbenzenemethanamine (Oya et al., 2002)), 4-[^{18}F]-ADAM or AFA (*N*,*N*-dimethyl-2-(2-amino-4-[^{18}F]fluorophenylthio)benzylamines (Shiue et al., 2003a)), [^{18}F]AFM (2-[2-(dimethylaminomethyl)phenylthio]-5-[^{18}F]fluoromethylphenylamine (Huang et al., 2005)) and 2-(2′-((Dimethylamino)methyl)-4′-(3-[^{18}F]fluoropropoxy)-phenylthio)benzenamine (Wang et al., 2008) have been synthesized and found to be potential SERT imaging agents. As no ^{18}F-labelled PET tracer for SERT imaging is currently available for clinical studies, validation of one of these ^{18}F analogs as a SERT imaging agent, namely 4-[^{18}F]-ADAM, has clinical significance.

In a preliminary study, we evaluated 4-[^{18}F]-ADAM as a potential SERT imaging agent in a healthy baboon (Shiue et al., 2003b). Based on these results, we aim to validate this finding in rats and further characterize this radioligand using both micro-PET imaging and autoradiography analysis of rats treated with paroxetine, a serotonin selective reuptake inhibitor (SSRI). In addition, we plan to study the sensitivity and specificity of 4-[^{18}F]-ADAM for SERT using a 5,7-dihydroxytryptamine (5,7-DHT)-lesioned rat model and a PCA-induced 5-HT depletion rat model.

Materials and methods

Radiopharmaceuticals and drugs

The paroxetine, PCA, and 5,7-DHT were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. The precursor, *N*,*N*-dimethyl-2-(2,4-dinitrophenylthio)benzylamine was synthesized as previously described (Shiue et al., 2003a; Peng et al., 2008).

The 4-[^{18}F]-ADAM was synthesized as previously reported (Shiue et al., 2003a). Briefly, aqueous [^{18}F]fluoride, produced via the ^{18}O(p, n)^{18}F nuclear reaction, was transferred through a QMA cartridge. The trapped [^{18}F]fluoride was eluted with a solution containing K_2CO_3 (5.2 mg) in H_2O (0.1 ml) and Kryptofix $_{2.2.2}$ (28.6 mg) in CH_3CN (0.9 ml). The solution was evaporated at 125 °C with a stream of helium and co-evaporated with CH_3CN (1 ml) to dryness. A solution of precursor (2–3 mg) in 0.5 ml of DMSO was added to the K[^{18}F]/K $_{2.2.2}$ residue. The solution was maintained at 125 °C for 5 min and then cooled to room temperature. Ten ml of water was added and the solution was passed through a C_{18} Sep-Pak. The crude intermediate product was eluted with 5 ml of CH_2Cl_2 and the solvent was evaporated to dryness. The residue was dissolved in 0.3 ml of ethanol and a saturated solution of $Cu(OAc)_2$ (~20 mg) in 1 ml of ethanol was added, followed by $NaBH_4$ (~9 mg) in 0.6 ml of ethanol. The mixture was heated at 80 °C for 20 min. The reaction mixture was filtered through a millipore filter (0.22 μm) by helium purge and then injected into a semi-preparative HPLC (10×250 mm, NUCLEOSIL 100-5 C_{18} Nautilus; CH_3CN:0.1 M HCO_2NH_4 (30:70) containing 0.3% (by volume) of acetic acid (4 ml/min). The effluent was passed through UV and radioactivity detectors. The fraction containing the product was collected in a vessel containing 200 ml of H_2O and then passed through a tC_{18} cartridge. The trapped product in the tC_{18} cartridge was eluted with 1 ml of ethanol into 10 ml of normal saline. The saline solution was passed through a millipore filter (0.22 μm) into a multi-injection vial. The radiochemical yield of 4-[^{18}F]-ADAM was 1.5±0.3% (n=13, EOS). The synthesis time was 120 min and the specific activity was 1.75±0.77 Ci/μmol (n=13, EOS).

Animal preparation and study design

All animal study protocols were approved by the institutional animal care and use committee. Fifty eight-week old male Sprague–Dawley rats weighing between 280 and 310 g (mean: 295±15 g) were housed in the animal facilities at the National Defence Medical Center (Taipei, Taiwan) at a constant temperature of 23±2 °C and a controlled light/dark cycle (light from 7:00 AM to 7:00 PM). Rats were maintained on a complete pellet diet and tap water for a period of 2 weeks prior to the studies.

Rats were randomly allocated to one of four study groups. Group 1 assessed the dynamic biodistribution of 4-[^{18}F]-ADAM over 2 h using micro-PET in normal control rats (n=5) to determine the time of maximal 4-[^{18}F]-ADAM brain binding. After the maximal specific binding time point was determined, the acquisition time was utilized for all subsequent studies. The autoragiography and the static micro-PET studies in normal rats (n=3) were also performed at the maximal specific binding time point. In group 2, the brain biodistribution of 4-[^{18}F]-ADAM was assessed in rats pre-treated with paroxetine (0.01 mg/kg, 0.05 mg, 0.1 mg/kg, or 0.25 mg/kg) using micro-PET and autoradiography (n=3 for each dosage). In group 3, the biodistribution of 4-[^{18}F]-ADAM was assessed in 5,7-DHT-lesioned rats (n=5) using micro-PET and autoradiography. In group 4, the distribution of 4-[^{18}F]-ADAM was assessed in rats (n=3) pre-treated with PCA using micro-PET and autoradiography.

Positron emission tomography

For the dynamic study of biodistribution of 4-[^{18}F]-ADAM, rats were anesthetized with an isoflurane/oxygen mixture (5% isoflurane for induction, and 2% for maintenance) and then injected with a bolus of 11.1–14.8 MBq (0.3–0.4 mCi) 4-[^{18}F]-ADAM via the tail vein. Fully three-dimensional list-mode emission data were immediately collected for 120 min using a micro-PET R4 scanner (Concorde MicroSystems, Knoxville, TN: energy window was 350–650 keV, timing window was 6 ns). Dynamic sinograms were produced with 12×10 s, 6×30 s, 5×300 s, 3×600 s and 4×900 s frames. The images were reconstructed by the Fourier rebinning and 2-D filtered back projection using a ramp filter with cutoff at Nyquist. All these processes were carried out by MicroPET Manager (version 2.3.3.6), provided by the manufacturer. The PET images were analyzed using ASIPro VM 6.3.3.1 software (Concorde MicroSystems, Knoxville, TN). A cylinder calibration method was used to convert the image units from cps per voxel (cps/voxel) to nCi per cm^3 (nCi/cm^3). Finally, post smoothing using Gaussian filters (transverse FWHM 3 mm and axial FWHM 1 mm) was performed to reduce image noise.

For static imaging, fully 3-dimensional list mode data were collected from 60–90 min after the radioligand injection and the data were processed as already above.

Volumes of Interest (VOI) were defined on reconstructed and summated PET-images according to the MRI and rat brain atlas