Nuclear Medicine and Biology 40 (2013) 879–886

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

Characterization of FlipIDAM as a SERT-selective SPECT imaging agent ☆

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ARTICLE INFO

Article history:
Received 17 August 2012
Revised in revised form 23 May 2013
Accepted 4 June 2013

Keywords:
SERT
SPECT
5-HTT
Brain imaging
Radioligand

ABSTRACT

Introduction: Biological evaluation of [^{125}I]FlipIDAM (2-((2-((dimethylamino)methyl)-4-iodophenyl)thio) phenyl)methanol ([^{125}I]**4**), a new single-photon emission computed tomography (SPECT) radioligand for imaging the serotonin transporter (SERT) which displayed improved in vivo kinetics for mapping SERT binding sites in the brain.

Methods: In vitro binding studies of [^{125}I]**4** were performed with membrane homogenates of LLC-PK1 cells stably transfected and overexpressing one of the monoamine transporter (SERT, DAT or NET) and rat cortical homogenates. Biodistribution and ex vivo autoradiography studies were carried out in rats. In vivo competition experiments were evaluated to determine the SERT selectivity of [^{125}I]**4** vs. [^{125}I]IDAM ([^{125}I]**1**).

Results: In vitro binding studies of **4** showed excellent binding affinity ($K_{i,SERT}$ = 0.90 ± 0.05 nM) and excellent selectivity over the other monoamine transporters (100 fold and >4000 fold for NET and DAT respectively). Scatchard analysis of saturation binding of [^{125}I]**4** to rat cortical homogenates gave a K_d value of 0.5 ± 0.09 nM and a B_{max} value of 801.4 ± 58.08 fmol/mg protein. The biodistribution study showed rapid high brain uptake (3.09 ± 0.11% dose/organ at 2 min) and a good target to non-target ratio (hypothalamus to cerebellum) at 30 min (2.62) compared to [^{125}I]**1** (2.19). Ex vivo autoradiography showed that FlipIDAM localizes in accordance with SERT distribution patterns in the brain. In vivo and ex vivo competition experiments with specific and non-specific SERT compounds also showed that [^{125}I]**4** binds specifically to SERT rich regions.

Conclusions: The biological evaluation of [^{125}I]**4** demonstrates that [^{123}I]**4** would be a good candidate for SPECT imaging of SERT.

1. Introduction

Serotonin (5-HT) is a neurotransmitter, found in the central nervous system (CNS), which regulates physiological functions, such as sleep, appetite and mood [1]. It is also implicated in the pathophysiology of neurodegenerative diseases such as Parkinson's disease (PD) or Alzheimer's disease (AD). The serotonin transporter (SERT) is found at the presynaptic level and in the cell bodies of 5-HT neurons in the brain [2]. SERT controls the concentration of 5-HT in the synaptic cleft, and the postsynaptic action, by transporting 5-HT back to the presynaptic neurons. Several lines of evidence suggest that patients suffering from mood disorders, such as depression, anxiety, schizophrenia or bipolar disorder, show a lower synaptic 5-HT concentration than seen in healthy people [3]. One common therapeutic approach is to increase the 5-HT concentration in the synaptic cleft. Selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine [4], paroxetine [5], sertraline [6], or escitalopram [7], target SERT and block its action. SSRIs are currently the most prescribed antidepressants in the world [8,9]. There are fewer side effects and interactions than there are with other drugs, such as tricyclic antidepressants, that target not only SERT but also the transporter of norepinephrine (NET), and to a lesser extent the dopamine transporter (DAT). Unfortunately, suicidal events are increased during the first ten days of treatment and not all patients respond to SSRIs [8,10]. These variations in response could be due to individual differences in the concentration of SERT in the brain [10]. Studies with tritiated SSRIs, such as imipramine [11], citalopram [12] or paroxetine [13], on human brain slices, have shown reduced binding in the brains of patients suffering from AD and PD [14], as well as for depressed [15] or suicidal patients [16], as compared to healthy brains.

Abbreviations: DASB, (N,N-dimethyl-2-(2-amino-4-cyanophenylthio)benzylamine); IACUC, Institutional Animal Care and Use Committee; (+)-McN5652, trans-1,2,3,5,6,10- < beta > -hexahydro-6-[4-(methylthio)phenyl-[pyrrolo-[2,1- < alpha >] isoquinoline; FlipIDAM (**4**), (2-((2-((dimethylamino)methyl)-4-iodophenyl)thio)phenyl)methanol); IDAM (**1**), (5-iodo-2-((2-((dimethylamino)methyl)phenyl)thio)benzyl alcohol); <beta > -CIT, 2 < beta > -carbomethoxy-3 < beta > -(4-iodophenyl) tropane; SPECT, single photon emission computed tomography; nor- < beta > -CIT, 2 < beta > -carbomethoxy-3 < beta > -(4-iodophenyl); SERT, serotonin transporter; SSRI, selective serotonin reuptake inhibitor; St, striatum; Th, thalamus; 5-HT, serotonin; ADAM (**2**), 2-((2-((dimethylamino)methyl)-phenyl)thio)-5-iodophenylamine; FlipADAM (**3**), 2-(2'-((dimethylamino)methyl)-4'-iodophenylthio)benzenamine; MADAM, N,N-dimethyl-2-(2-amino-4-methylphenylthio)benzylamine.

☆ Financial support: This work was supported by a grant awarded by the National Institutes of Health (ROI-MH068782, H.F.K.).

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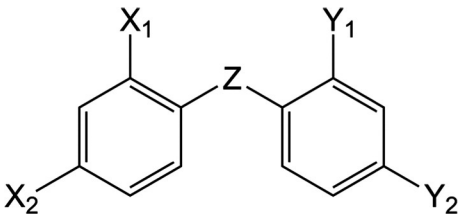
0969-8051/$ – see front matter © 2013 Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.nucmedbio.2013.06.001

SERT imaging agents could be utilized in a variety of ways to increase our understanding of disease states and improve patient care. Imaging SERT distribution in vivo would enable clinicians to compare the concentration and distribution of SERT in the brains of patients suffering from depression or degenerative disease with that in the brains of healthy patients. The ability to measure drug occupancy might shed light on the difference between responders and non-responders to SSRI treatment. Physicians might also be able to adapt the treatment to provide the minimum dose necessary to reach >60–70% occupancy and thereby reduce side effects [17]. Moreover, in vivo localization of the SERT present on 5-HT neurons would allow clinicians to assess the integrity of the neurons.

Several radioligands have already been developed for imaging SERT with PET and SPECT. PET imaging agents exist for clinical studies like [^{11}C]McN5652 [18], and [^{11}C]DASB [19]. The [^{11}C]DASB is most commonly used in clinics for PET imaging of SERT [20] with an excellent binding affinity (Ki = 0.97 nM) [21]. There have also been attempts to develop SPECT ligands. SPECT resolution is lower than that of PET, but SPECT has different advantages, such as lower cost, a longer half-life of the radioisotopes and no need for a cyclotron. The first potential SPECT ligand was β-[^{123}]CIT [22] and its derivative nor-β-[^{123}]CIT [23]. The diarylsulfides family, derived from 403U76 [24] which was reported by GlaxoWellcome as an antidepressant and anxiolytic with an excellent affinity for NET and SERT, inspired many potent SERT ligands, such as ADAM (**2**) [25], IDAM (**1**) [26], DASB [19] and MADAM [27] (see Table 1). Among these diarylsulfide compound derivatives, [^{123}I]**2** [25] and [^{123}I]**1** [26] were well suited for SPECT imaging. Both have excellent selectivity and target to non-target ratios in studies conducted in non-human primate brains. [^{123}I]**2** has become the leading agent for clinical SPECT imaging and has been shown to be safe for human studies [28]. [^{123}I]**2** has been used to estimate SERT occupancy of SSRIs in clinical disorders, such as depression, by comparing healthy volunteers and depressed patients treated with an SSRI [29]. It has also been used in studies of migraines [30], borderline personality disorder [31] and eating disorders like bulimia [32] or night eating syndrome [33]. While [^{123}I]**2** is useful, there is room for improvement. A study has questioned its specificity of binding by showing variability of the estimated occupancy values [34]. More importantly, it has very slow brain kinetics. A study by Sacher et al. in 2006 showed that for optimum imaging results, the SPECT scan should be done 205 to 320 min after the injection of [^{123}I]**2** [35].

In order to address the above problems with [^{123}I]**2**, we have synthesized several PET radioligand derivatives of ADAM. The most potent was 4-[^{18} F]**2** (see Table 1), which shows conclusive results both in rats [36] and in non-human primates [37] and should be useful for human brain studies. However, this tracer is not optimal for clinical use because it reaches its equilibrium 120 to 150 min after injection. In another iteration, we made a structural change to ADAM by switching iodine from 5-position at phenylring A to 4'-position at phenylring B. The new ligand, [^{125}I]FlipADAM (**3**), showed an enhanced target to non-target ratio at 30 min and faster kinetics than [^{123}I]**2** and [^{123}I]**1** [38]. In view of these encouraging results, we decided to make a similar structural change to IDAM. This study is a biological characterization of the new ligand, FlipIDAM, [^{125}I]**4**.

Table 1
Series of compounds synthesized based on the same core structure.



Compound		X1	X2	Y1	Y2	Z
IDAM	1	CH2OH	I	CH2N(CH3)2	H	S
ADAM	2	NH2	I	CH2N(CH3)2	H	S
FlipADAM	3	NH2	H	CH2N(CH3)2	I	S
FlipIDAM	4	CH2OH	H	CH2N(CH3)2	I	S
ODAM	5	CH2OH	I	CH2N(CH3)2	H	O
6		CH2OH	I	CH2NH(CH3)	H	S
7		CH2OH	CI	CH2N(CH3)2	H	S
8		CH2NH(CH3)	I	CH3	H	NH
9		H	I	CH2N(CH3)2	H	O
10		H	I	CH2N(CH3)2	H	CH2
DASB		NH2	CN	CH2NH2	H	S
MADAM		NH2	CH3	CH2N(CH3)2	H	S
4-F-ADAM		NH2	F	CH2N(CH3)2	H	S

2. Materials and methods

Adult male Sprague Dawley rats (250–275 g; Charles River Laboratories, Malvern, PA) were used for the experiments. Animals were kept in the animal facility (room temperature 22 °C with a 12 h day–night cycle). All animal experiments were performed in accordance with the NIH guide for the Care and Use of animals. All protocols were approved by The Institutional Animal Care and Use Committee (IACUC, University of Pennsylvania).

All reactions were carried out under an atmosphere of argon in oven-dried glasswares. Anhydrous acetone, N,N-dimethylformamide, as well as other reagents were used as they were received from commercial source. Chromatographic separations were carried out using Sigma-Aldrich silica gel (230–400 mesh) or CombiFlash® Rf 200 automated chromatography system (Teledyne Isco) with HP Silica Chromatography Columns (12 g) (Teledyne Isco). Analytical thin layer chromatography was performed on EM reagent 0.25 nm silica gel 60-F plates with F-254 indicator. ^{1}H-NMR spectra were recorded on a Bruker DPX-200 (200 MHz) and are reported in ppm (δ) from residual solvent peaks ($CDCl_3$: δ 7.26 ppm). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublet, ddd = doublet of doublet of doublet), coupling constants (Hz) and integration. ^{13}C-NMR spectra were recorded on a Bruker DPX-200 (50 MHz) spectrometer with complete proton decoupling. Chemical shifts are reported in ppm from residual solvent resonance as the internal standard ($CDCl_3$: δ 77.0 ppm). HRMS was performed on an LC/MSD TOF (Agilent Technologies). ODAM (**5**) was synthesized according to literature [39]. Ligands **6** to **10** were synthesized in our laboratory (unpublished results). Citalopram and GBR12909 were purchased from TOCRIS-Bioscience (Minneapolis, MN, USA). **1** was synthesized by SRI international (Menlo Park, CA, USA). Nisoxetine and Bovin Serum Albumin (BSA) were obtained from Sigma (St. Louis, MO, USA). Isoflurane anesthesia was purchased from Butler Schein (Isothesia, Dublin, OH, USA).

2.1. Synthesis and radiolabeling

2.1.1. Preparation of **11**, the tin labeling precursor

50 mg of FlipIDAM (**4**) [40] was added to tetrakis (triphenylphosphine) palladium (14.5 mg, 0.012 mmol), bis(tributyltin) (330 μL, 0.625 mmol) and triethylamine (1.0 mL). The resulting mixture was purged with argon and reacted at 120 °C for 20 min in a microwave reactor. Triethylamine was removed under vacuo and the resulting mixture was purified by CombiFlash automated chromatography system (EtOAc/hexanes, 0% to 100%). The desired tin precursor (**11**) was isolated as a yellow liquid (53 mg, 75% yield, 98% HPLC purity). **11** was further purified by high-performance liquid chromatography (HPLC) Phenomenex Gemini C18 semi-prep column (250 × 10 mm, 10 μm) with 20 mM ammonium formate buffer/CH_3CN under