Nuclear Medicine and Biology 54 (2017) 34–40

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

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

[^{11}C]AZ10419096 – a full antagonist PET radioligand for imaging brain 5-HT$_{1B}$ receptors

Anton Lindberg [a,*], Sangram Nag [a], Magnus Schou [a,b], Akihiro Takano [a], Junya Matsumoto [a], Nahid Amini [a], Charles S. Elmore [c], Lars Farde [a,b], Victor W. Pike [d], Christer Halldin [a]

[a] Department of Clinical Neuroscience, Center for Psychiatry Research, Karolinska Institutet and Stockholm County Council, SE-171 76 Stockholm, Sweden
[b] AstraZeneca, Personalised Healthcare and Biomarkers, AstraZeneca PET Science Centre, Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[c] Isotope chemistry, Early Chemical Development, Pharmaceutical Sciences Innovative Medicines and Early Development, AstraZeneca, Mölndal, Sweden
[d] Molecular Imaging Branch, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20892, USA

ARTICLE INFO

Article history:
Received 26 April 2017
Received in revised form 5 June 2017
Accepted 14 July 2017

Keywords:
5-HT$_{1B}$ receptor
Antagonist
Radioligand
Carbon-11
PET

ABSTRACT

Introduction: The serotonergic system is widely present in all regions of the central nervous system (CNS) and plays a key modulatory role in many of its functions. Positron emission tomography (PET) is used to study several serotonin receptors in CNS *in vivo*. The G-protein coupled receptor 5-HT$_{1B}$ is mostly present in the occipital cortex and in midbrain and is linked to several psychiatric disorders. There is evidence that agonist PET radioligands for neuroreceptors are more sensitive to endogenous neurotransmitters than antagonists. Our previously developed 5-HT$_{1B}$ receptor PET radioligand, [^{11}C]AZ10419369, is now considered a partial agonist. In this work we are aiming to develop a full antagonist PET radioligand for imaging brain 5-HT$_{1B}$ receptors, and evaluate its sensitivity to increased endogenous serotonin concentration.
Materials: [^{11}C]AZ10419096 was synthesized by rapid methylation of the prepared corresponding *N*-desmethyl precursor with [^{11}C]methyl triflate. Five PET measurements were performed in cynomolgus monkeys, consisting of two at baseline, one after treatment of a monkey with a 5-HT$_{1B}$ antagonist, AR-A000002, and two in which fenfluramine was administered during scanning to induce endogenous serotonin release.
Results and discussion: [^{11}C]AZ10419096 was synthesized in high yield and purity within 30 min, including purification, formulation and sterile filtration. The baseline PET measurements demonstrated [^{11}C]AZ10419096 to have favorable radioligand characteristics, including high specific binding in brain regions that have high 5-HT$_{1B}$ density, such as occipital cortex and globus pallidus, as well as subsequent rapid elimination from brain and a minor abundance of lipophilic radiometabolites in plasma. AR-A00002 completely blocked radioligand receptor-specific binding. Fenfluramine produced a distinct displacement of radioligand consistent with an expected increase of synaptic endogenous serotonin concentration.
Conclusions: [^{11}C]AZ10419096, a full 5-HT$_{1B}$ antagonist PET radioligand, demonstrates high specific binding in monkey brain that is sensitive to competition from a known 5-HT$_{1B}$ antagonist as well as to putatively increased endogenous serotonin levels.

Published by Elsevier Inc.

1. Introduction

The serotonergic (5-HT) system originates from the brain stem and projects widely into the central nervous system (CNS). This system has a key modulatory role for several basic physiological brain functions and is of major interest in research on the pathophysiology and treatment of neuropsychiatric disorders, such as depression and anxiety [1]. Each of the fourteen different 5-HT receptor subtypes has a distinct distribution in brain. Given its implication in the treatment of depression and anxiety, the 5-HT$_{1B}$ receptor has long been pursued as a target for drug development [2]. These efforts have led to the successful development of [^{11}C]AZ10419369 (K_D = 0.44 nM) [3,4] and [^{11}C]P943 (K_D = 1.22 nM) [5,6] (Fig. 1), as the first effective radioligands for imaging of the 5-HT$_{1B}$ receptor in the human brain with positron emission tomography (PET).

An interesting application of PET in neuroscience research is the non-invasive investigation of drug-induced changes in synaptic neurotransmitter concentrations [7]. Although this experimental paradigm has been applied for several receptor systems, the fundamental prerequisites for such studies at a molecular level remain to be fully understood. In several studies of the neurotransmission systems that have been examined so far the radioligands target primarily postsynaptic receptors, such as the antagonists [^{11}C]raclopride and [^{11}C]FLB 457 for the

* Corresponding author.
E-mail address: anton.lindberg@ki.se (A. Lindberg).

http://dx.doi.org/10.1016/j.nucmedbio.2017.07.007
0969-8051/Published by Elsevier Inc.

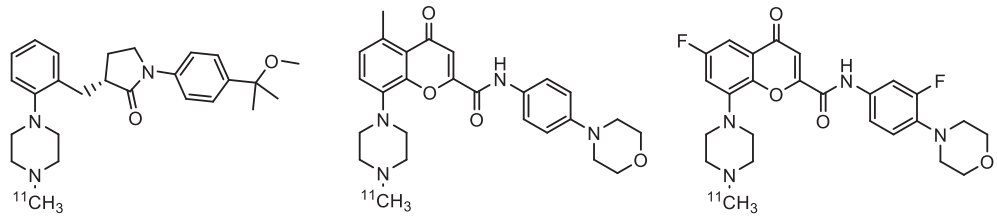

Fig. 1. Structures of [^{11}C]P943, [^{11}C]AZ10419369 and [^{11}C]AZ10419096. The K_D value for P943 is taken from the NIMH Psychoactive Drug Screening Program, and those for AZ10419369 and AZ10419096 were provided by AstraZeneca.

D_2 dopamine receptor [8,9] and [^{11}C]ORM-13070 for α_{2c}-adrenoceptors [10,11]. It has been hypothesized that radioligands that dissociate rapidly from the targeted receptor will exhibit greater sensitivity to changes in endogenous neurotransmitter concentrations [12]. Agonists are generally expected to dissociate more rapidly than antagonists because of the rapid G-protein decoupling expected on binding of a full agonist to a G-protein coupled receptor; moreover, an agonist should only bind to the sub-set of receptors that are G-protein coupled, whereas an antagonist should bind to a receptor irrespective of whether it is actually G-protein coupled [13]. For the dopaminergic system, agonist radioligands, such as [^{11}C]MNPA, have been found more sensitive to endogenous neurotransmitter concentrations than antagonists, such as [^{11}C]raclopride [14,15]. We are interested in investigating if any such difference can be observed for radioligands targeting G-protein-coupled 5-HT_{1B} receptors.

The 5-HT_{1B} receptor is partly expressed presynaptically where it serves as an autoreceptor regulating the release of serotonin. The 5-HT_{1B} receptor PET radioligand [^{11}C]AZ10419369 has been shown to be sensitive to the synaptic 5-HT concentration in NHP and humans [12,16]. This ligand was originally described as a full antagonist, but more recent *in vitro* data indicate that this ligand is a partial agonist (K_D = 0.44 nM, antagonist GTPγS effect 48%; agonist GTPγS effect 53.2%; 5-HT_{1B} IC_{50}: 50 nM).

In relation to this updated view of AZ10419369, we intended to explore how the intrinsic activity of a 5-HT_{1B} PET radioligand affects sensitivity to endogenous 5-HT. Thus, from a library of over 3000 compounds, we identified the 5-HT_{1B}-specific high-affinity full antagonist AZ10419096 (K_D = 0.13 nM, antagonist GTPγS effect 190%; 5-HT_{1B} IC_{50}: 2 nM; 5-HT_{1A} IC_{50}: 686 nM; 5-HT_{1D} IC_{50}: 5 nM) as a prospective full antagonist PET radioligand for the 5-HT_{1B} receptor. The aim of the present study was to label AZ10419096 with carbon-11 ($t_{1/2}$ = 20.4 min) for preliminary evaluation as a PET radioligand for imaging of 5-HT_{1B}-receptor binding in the cynomolgus monkey brain.

2. Material and methods

2.1. General

Reference AZ10419096 was provided by AstraZeneca Pharmaceuticals, Mölndal, Sweden. All other chemicals were obtained from commercial sources and were used as received.

2.2. Chemistry

6-Fluoro-N-(3-fluoro-4-morpholinophenyl)-4-oxo-8-(piperazin-1-yl)-4H-chromene-2-carboxamide ((*N*-desmethyl)AZ10419096). AZ10419096 (50 mg, 0.10 mmol) was taken up in dichloroethane (5 mL) and 1-chloroethylchloroformate (22 mg, 0.15 mmol) was added. The reaction mixture was then heated to reflux, stirred overnight and then concentrated *in vacuo*. The intermediate product was isolated by silica gel chromatography (2 − 7% MeOH in DCM). Fractions containing the desired intermediate were pooled and concentrated *in vacuo*, and then taken up in MeOH (5 mL) and heated to reflux for 1 h. The solvent was removed *in vacuo* to give the desired product (*N*-desmethyl)AZ10419096; 17 mg, 0.036 mmol, 36%. ^{1}H NMR (400.03 MHz, MeOD) δ: 7.66 (dd, 1H), 7.46 (m, 2H), 7.32 (dd, 1H), 7.09 (m, 2H), 3.86 (t, 4H), 3.54 (d, 8H), 3.09 (t, 4H). ^{13}C NMR (151 MHz, DMSO) δ 42.77, 47.08, 50.59 (J = 3.0), 66.14, 101.39 (J = 24.4), 108.80 (J = 25.7), 110.31, 111.26, 116.78, 119.08, 125.34, 132.58, 136.52 (J = 8.6), 143.10 (J = 9.7), 145.14, 154.14 (J = 243.7), 154.98, 157.89, 159.38 (J = 244.8), 177.05. HRMS (ESI+) *m/z* calculated 471.1838, found 471.1821. Product was analyzed with HPLC to confirm absence of starting material.

2.3. Radiochemistry

[^{11}C]Methane was prepared through the ^{14}N(p,α)^{11}C reaction by bombarding a mixture of nitrogen and hydrogen (10%) gas with a beam of 16.7 MeV protons produced from a biomedical cyclotron (GE PETtrace; Uppsala, Sweden). [^{11}C]Methane was converted into [^{11}C]methyl iodide by high temperature iodination in a gas-phase recirculation system and subsequently transformed into [^{11}C]methyl triflate, as described previously [17,18]. [^{11}C]AZ10419096 was obtained by trapping [^{11}C]methyl triflate in a solution of *N*-desmethyl AZ10419096 (0.1 − 0.3 mg) and sodium hydroxide (0.1 M, 6 μL) in acetone (400 μL) at room temperature (Fig. 2). The sealed reaction vial was left for 2 min. The reaction mixture was injected onto a reversed phased HPLC column (ACE C-18; 250 × 10 mm; ACE) eluted with MeCN: HCO_2NH_4 (0.1 M) (40: 60 v/v) containing sodium ascorbate (0.01%) at 6 mL/min. Eluate was monitored in series for absorbance at (254 nm) and radioactivity (Geiger–Müller tube). The fraction containing [^{11}C]AZ10419096 (t_R = 6 − 8 min) was collected, evaporated to dryness, formulated in saline (pH = 6; 4 − 6 mL), and finally filtered through a sterile filter (0.22 μm; Millipore Millex®GV) into a sterile vial.

The radiochemical purity of the product was determined with reversed phase HPLC on an Advanced chromatography technologies Ltd ACE 5 C18-HL column (C18, 3.9 Ø × 300 mm, 10 μm particle size), with eluate monitored in series for absorbance at 254 nm and radioactivity (β-flow detector; Beckman, Fullerton, CA). For the analysis, the column was eluted at 3 mL/min with a gradient of 10 − 90% MeCN in HCO_2NH_4 (0.1 M) for 10 min. [^{11}C]AZ10419096 (t_R of 6 − 7 min) was identified by its co-mobility when coinjected with reference AZ10419096.

The specific radioactivity (SA) of the final product was measured with HPLC under the conditions described above for radiochemical analysis. The UV absorbance (λ = 254 nm) response was calibrated for mass of ligand and calculated as the radioactivity of the radioligand (GBq) divided by the amount of the associated carrier substance (μmol). Each sample was analyzed three times and compared to a reference standard also analyzed three times.