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Carbon-11 HOMADAM: A novel PET radiotracer for imaging serotonin transporters

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Abstract

Carbon-11-labeled *N*,*N*-dimethyl-2-(2′-amino-4′-hydroxymethylphenylthio)benzylamine (HOMADAM) was synthesized as a new serotonin transporter (SERT) imaging agent.

Methods: Carbon-11 was introduced into HOMADAM by preparation of *N*-methyl-2-(2′-amino-4′-hydroxymethylphenylthio)benzylamine followed by alkylation with carbon-11 iodomethane. Binding affinities of HOMADAM and the radiolabeling substrate, *N*-methyl-2-(2′-amino-4′-hydroxymethylphenylthio)benzylamine, were determined in cDNA transfected cells expressing human SERT, dopamine transporters (DAT) and norepinephrine transporters NET using [^{3}H]citalopram, [^{125}I]RTI-55 and [^{3}H]nisoxetine, respectively. MicroPET brain imaging was performed in monkeys. Arterial plasma metabolites of HOMADAM were analyzed in a rhesus monkey by high-performance liquid chromatography (HPLC).

Results: HOMADAM displayed high affinity for the SERT (K_i=0.6 nM). *N*-methyl-2-(2′-amino-4′-hydroxymethylphenylthio)benzylamine displayed moderate affinity for the SERT (K_i=15.11 nM). The affinities of HOMADAM for the DAT and NET were 2000- and 253-fold lower, respectively, than for the SERT. [^{11}C]HOMADAM was prepared from [^{11}C]iodomethane in approximately 25% radiochemical yield (decay-corrected to end of bombardment). MicroPET brain imaging studies in monkeys demonstrated that [^{11}C]HOMADAM uptake was selectively localized in the midbrain, thalamus, pons, caudate, putamen and medulla. The midbrain-to-cerebellum, pons-to-cerebellum, thalamus-to-cerebellum and putamen-to-cerebellum ratios at 85 min were 4.2, 2.8, 2.3 and 2.0, respectively. HOMADAM binding achieved quasi-equilibrium at 45 min. Radioactivity in the SERT-rich regions of monkey brain was displaceable with *R*,*S*-citalopram. Radioactivity in the DAT-rich regions of monkey brain was not displaceable with the DAT ligand RTI-113. Radioactivity in the SERT-rich regions of monkey brain was displaceable with the *R*,*S*-reboxetine, a NET ligand with a high nanomolar affinity for SERT. Arterial plasma metabolites of HOMADAM were analyzed in a rhesus monkey by HPLC and displayed a single peak that corresponded to unmetabolized HOMADAM.

Conclusion: HOMADAM is an excellent candidate for PET primate imaging of brain SERTs.

Keywords: Carbon-11; HOMADAM; *N*, *N*-dimethylbenzylamine; Serotonin transporter; MicroPET

1. Introduction

Many neuropsychiatric disorders such as depression [1], Alzheimer's disease [2], Parkinson's disease [3,4] as well as the neurotoxic effects of illicit drugs (e.g., MDMA) [5] involve abnormalities within the brain's serotonin system. Serotonergic neurons originate primarily from neuronal cell bodies of the medial and dorsal raphe in the brainstem and innervate widespread areas that include the hypothalamus, thalamus, striatum and cerebral cortex [6–8]. The serotonin transporter (SERT) is a protein residing on the presynaptic serotonergic neuron that regulates serotonin neurotransmission by removing released serotonin from the extracellular space back into the presynaptic neuron. Because of its localization, the SERT is a specific marker for the integrity and number of presynaptic terminals of serotonin-producing neurons [9–12]. When combined with PET, radioligands

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binding specifically to the SERT are potentially useful as in vivo biochemical probes for quantitating the brain regional distribution of SERT and understanding how alterations in SERT are related to these various disorders and defining in vivo interaction of SERT antagonists commonly used to treat mood and anxiety disorders, with this molecular site of drug action.

A number of radioligands for PET have been prepared and evaluated for imaging the SERT in the central nervous system (CNS). Several classes of candidate compounds have been screened for their SERT affinity, such as selective serotonin reuptake inhibitors [13–20] phenyl nortropane and pyrroloisoquinoline derivatives. Most of them appeared to be unsuitable for imaging due to high lipophilicity and/or high nonspecific binding and/or inappropriate in vivo kinetics. The ligand (+)McN5652, from the pyrroloisoquinoline series, has also been radiolabeled with carbon-11 and studied in humans. This radioligand has significant limitations such as modest signal to background due to high nonspecific binding [21,22]. In the phenyl nortropane series, only ZIENT radiolabeled with iodine-123 and carbon-11 and ZIET radiolabeled with carbon-11 displayed high specific binding at the SERT with a low nonspecific accumulation and have been proposed as potential radioligands for SPECT and PET imaging, respectively, for SERT [20,23,24].

More recently, a new class of radiotracers based on a diarylsulfide motif has been introduced such as [^{123}I]ADAM, [^{11}C]DASB, [^{11}C]AFM, [^{11}C]EADAM, [^{11}C]MADAM, [^{11}C]DAPP and [^{11}C]DAPA (Fig. 1). ADAM has been labeled with iodine-123 and showed promise as new SPECT imaging agent for the SERT [25–27]. [^{11}C]ADAM was also prepared and evaluated as a PET SERT radioligand [28]. However, the slow binding kinetics displayed by [^{11}C]ADAM were found to be inappropriate for the short half-life of carbon-11. In attempts to develop a SERT imaging agent with kinetic properties compatible with the 20-min half-life of carbon-11, several new candidate *N*-[^{11}C]methyl-*N*′-methyl-2-(2′-amino-4′-substituted-phenylthio)benzylamines were synthesized and evaluated as PET SERT radioligands. PET imaging with [^{11}C]DAPP [29] or [^{11}C]DASB [29,30] in humans and with [^{11}C]DASB [31], [^{11}C]MADAM [32], [^{11}C]AFM [31,33], [^{11}C]DAPA [31,34] and [^{11}C]EADAM [35] in nonhuman primates displayed good specific-to-nonspecific (cerebellum) ratios for the SERT-rich diencephalon (2:1–3.5:1). PET imaging studies in baboons with [^{11}C]ADAM, [^{11}C]DAPA, [^{11}C]DASB and [^{11}C]AFM [31,34] demonstrated that only [^{11}C]DASB reached a state of quasi-equilibrium binding (a condition where the ratio of radioactivity uptake in the region of interest (ROI) to reference region stays relatively constant) in the thalamus within 40 min postinjection. Fitted time–activity curves between thalamus and cerebellum satisfied this condition and supported [^{11}C]DASB as a preferred radioligand for imaging SERT sites by PET. [^{11}C]AFM displayed the highest specific-to-nonspecific ratio in the thalamus (3.5) but did not achieve a state of quasi-equilibrium binding in the thalamus by 90 min postinjection. In an effort to develop a carbon-11-labeled benzylamine displaying a significantly shorter time to attain quasi-equilibrium SERT binding than [^{11}C]AFM but comparable specific-to-nonspecific ratios in SERT-rich tissues, we prepared and evaluated a new derivative of the benzylamine series, carbon-11-labeled *N*, *N*-dimethyl-2-(2′-amino-4′-hydroxymethylphenylthio)-benzylamine (HOMADAM). In this report, we describe the radiosynthesis of [^{11}C]HOMADAM and the evaluation of its SERT imaging properties through in vitro competitive binding assays, lipophilicity measurements, metabolite analysis and a comparison with [^{11}C]DASB and [^{11}C]AFM by microPET imaging in macaque monkeys.

2. Materials and methods

2.1. General

All chemicals and solvents were analytical grade and were used without further purification. Thiosalicylic acid (**4**) was purchased from Sigma-Aldrich. The carbon-11 iodomethane ([^{11}C]CH_3I) was produced with a GE PET trace system (General Electric) from carbon-11 carbon dioxide ([^{11}C]CO_2) produced by a Siemens RDS 112, 11-MeV cyclotron. The melting points were determined in capillary tubes using an electrothermal apparatus and are uncorrected.

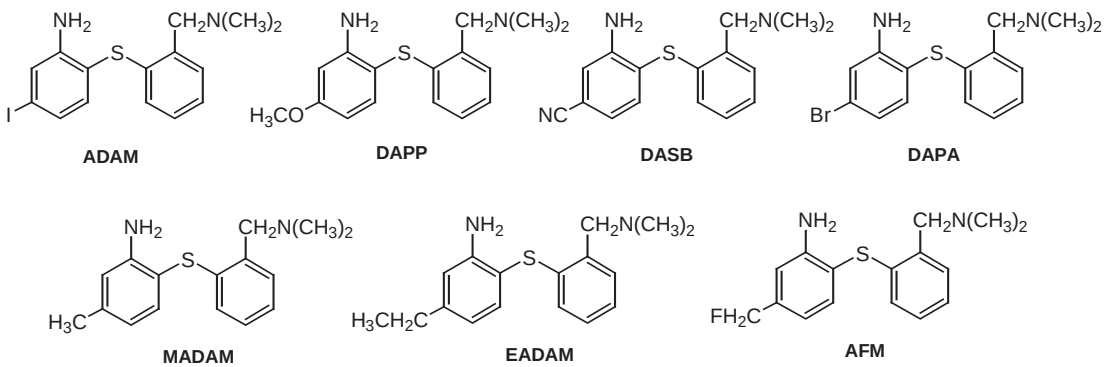

Fig. 1. Substituted benzylamines.