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ScienceDirect
Nuclear Medicine and Biology 35 (2008) 185–195
NUCLEAR MEDICINE — AND — BIOLOGY
www.elsevier.com/locate/nucmedbio

A new ^{18}F-labeled fluoroacetylmorpholino derivative of vesamicol for neuroimaging of the vesicular acetylcholine transporter☆

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Received 11 July 2007; received in revised form 24 August 2007; accepted 10 October 2007

Abstract

With the aim of producing selective radiotracers for in vivo imaging of the vesicular acetylcholine transporter (VAChT) using positron mission tomography (PET), here, we report synthesis and analysis of a new class of conformationally constrained vesamicol analogues with moderate lipophilicity. The sequential ring opening on *trans*-1,4-cyclohexadiene dioxide enabled an approach to synthesize 6-arylpiperidino-octahydrobenzo[1,4]oxazine-7-ols [morpholino vesamicols]. The radiosynthesis of the [^{18}F]fluoroacetyl-substituted derivative ([^{18}F]FAMV) was achieved starting from a corresponding bromo precursor [2-Bromo-1-[7-hydroxy-6-(4-phenyl-piperidin-1-yl)-octahydro-benzo[1,4] oxazin-4-yl]-ethanone] and using a modified commercial computer-controlled module system with a radiochemical yield of 27±4%, a high radiochemical purity (99%) and a specific activity of 35 GBq/μmol. In competitive binding assays using a PC12 cell line overexpressing VAChT and [^{3}H]-(−) vesamicol, 2-fluoro-1-[7-hydroxy-6-(4-phenyl-piperidin-1-yl)-octahydro-benzo[1,4]oxazin-4-yl]-ethanone (FAMV) demonstrated a high selectivity for binding to VAChT (K_i: 39.9±5.9 nM) when compared to its binding to sigma$_{1/2}$ receptors (K_i>1500 nM). The compound showed a moderate lipophilicity (log$D_{(pH\ 7)}$=1.9) and a plasma protein binding of 49%. The brain uptake of [^{18}F]FAMV was about 0.1% injected dose per gram at 5 min after injection and decreased continuously with time. Notably, an increasing accumulation of radioactivity in the lateral brain ventricles was observed. After 1 h, the accumulation of [^{18}F]FAMV, expressed as ratio to the cerebellum, was 4.5 for the striatum, 2.0 for the cortical and 1.5 for the hippocampal regions, measured on brain slices using ex vivo autoradiography. At the present time, 75% of [^{18}F]FAMV in the plasma was shown to be metabolized to various hydrophilic compounds, as detected by high-performance liquid chromatography. The degradation of [^{18}F]FAMV was also detected in brain extracts as early as 15 min post injection (p.i.) and increased to 50% at 1 h postinjection. In conclusion, although the chemical properties of [^{18}F]FAMV and the selectivity of binding to VAChT appear to be promising indicators of a useful PET tracer for imaging VAChT, a low brain extraction, in combination with only moderate specific accumulation in cholinergic brain regions and an insufficient in vivo stability prevents the application of this compound for neuroimaging in humans.

Keywords: Vesamicol; Radiosynthesis; Rat; Affinity; Selectivity; VAChT; Biodistribution; Cholinergic neurons

1. Introduction

The role of the cholinergic system in Alzheimer's disease (AD) has become increasingly important, since there has been a recognition of interrelationships between an impaired cortical cholinergic function and other pathological features of the disease, such as β-amyloid formation/deposition and local inflammatory up-regulation [1,2]. Early studies on postmortem brains of patients with AD had shown that a loss of cholinergic neurons in the basal forebrain [3] was associated with a decreased activity of choline acetyltransferase (ChAT), the biosynthetic enzyme for acetylcholine, in that region and also in cortical target areas [4–6]. The gene

☆ This work was supported by a grant from Sächsisches Ministerium für Wissenschaft und Kunst, contract no. 7531.50-03-0361-01/6.
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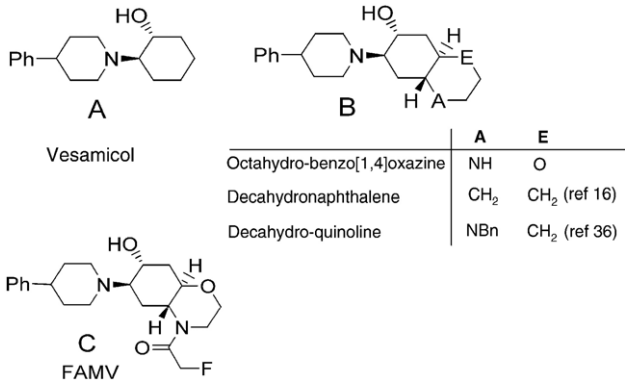
doi:10.1016/j.nucmedbio.2007.10.004

encoding ChAT is closely linked with the gene for the vesicular acetylcholine transporter (VAChT), where the entire VAChT coding region is localized within the first intron of the ChAT gene. This unique genetic organization is commonly referred to as the "cholinergic gene locus" [7–9]. This unique feature permits coordinated expression of the two genes throughout the adult nervous system and during development [7,10]. Hence, there is evidence from autoradiographic studies that the VAChT density is decreased in patients with AD [11]. Studies in rats treated with β-amyloid peptide indicate that an observed reduction in VAChT density is related to the memory impairment assumed to be induced by β-amyloid [12]. These studies were performed with ^{3}H-labeled phenylpiperidinyl cyclohexanol (vesamicol), which binds with high affinity to an allosteric binding site on the VAChT protein [13–16], thus providing the potential possibility to use this compound and its other radiolabeled derivatives for measuring cholinergic neuronal changes in vitro and in vivo.

In rats, [^{3}H]vesamicol [17], [^{125}I]iodobenzovesamicol (IBVM) [18] and [^{123}I]IBVM [19], were used for autoradiographic studies which indicated a cholinergic synaptic loss in the cortex and the hippocampus caused by a specific cholinergic lesion in the basal forebrain. In studies on postmortem human brain tissue of patients with AD, a dissociation between ChAT activity and [^{3}H]vesamicol binding to VAChT was observed [11,20,21]. When the more specific radioligand (+) [^{125}I]iodobenzyltrozamicol was used, a correlation of r=.72 between both cholinergic parameters was found [22]. The first and so far the only application for human in vivo VAChT brain imaging has been reported using 5-[^{123}I]iodobenzovesamicol [23]. Moreover, in patients with AD and Parkinson's disease a decreased cortical binding of this radioligand was reported [24].

Despite these efforts radiochemists are still confronted with the clinical demand to develop a PET radioligand appropriate to image the presynaptic cholinergic functionality in vivo [25]. Detailed in vitro analyses performed for a large number of vesamicol derivatives revealed their high affinity binding to VAChT but a lack of selectivity. More importantly, the binding to sigma receptors, which are widely distributed in the brain, seems to represent the main problem to be solved when new radioligands for VAChT binding are developed [26,27]. Because of the unique vesamicol binding site on VAChT, efforts are underway to synthesize new derivatives based on the vesamicol structure [28–34].

It was the intention of this study to develop a structurally modified vesamicol analogue which can be easily labeled with ^{18}F and presents a high selectivity in binding to VAChT. We aimed at a compound of moderate lipophilicity distinguished by a sufficient penetration of the blood–brain barrier and a specific accumulation in brain regions of high cholinergic densities. Based on these requirements, a novel class of conformationally fixed vesamicol analogues was synthesized by a sequential ring opening approach, starting



	A	E
Octahydro-benzo[1,4]oxazine	NH	O
Decahydronaphthalene	CH_2	CH_2 (ref 16)
Decahydro-quinoline	NBn	CH_2 (ref 36)

Fig. 1. Structures of vesamicol (A) and its two derivatives (B and C) used for radiotracer development for neuroimaging of VAChT.

from *trans*-1,4-cyclohexadiene dioxide [35]. The synthesized 6-amino-octahydrobenzo[1,4]oxazine-7-ol scaffold closely resembles to the decahydronaphthalene and decahydroquinoline substructure, both of which have been used recently as constituents of highly potent VAChT ligands (Fig. 1B) [16,36].

Here, we report on a fluoroacetyl-octahydro[1,4]benzoxazine derivative of vesamicol [2-fluoro-1-[7-hydroxy-6-(4-phenyl-piperidin-1-yl)-octahydro-benzo[1,4]oxazin-4-yl]-ethanone (FAMV)] (Fig. 1C) including (i) the radiosynthesis of its ^{18}F-labeled derivative, (ii) its binding affinity and specificity to VAChT in vitro, (iii) its in vivo distribution and rat brain uptake as well as (iv) its metabolism in the blood and the brain.

2. Materials and methods

2.1. Chemistry

A method to synthesize the parent secondary amine (Fig. 2, compound **5**) was described in detail elsewhere [35]. Briefly, it involves a sequential ring opening of diepoxide (**1**) with 4-phenylpiperidine (**2**) to obtain the monoepoxide (**3**) which was converted via a six-step sequence to racemic octahydro-6-(4-phenylpiperidin-1-yl)-2*H*-benzo[b][1,4]oxazin-7-ol (**5**). Both acyl derivatives [FAMV and 2-Bromo-1-[7-hydroxy-6-(4-phenyl-piperidin-1-yl)-octahydro-benzo[1,4]oxazin-4-yl]-ethanone (BrAMV)] were obtained from **5**, and the corresponding acyl halide was synthesized by applying a standard acylation procedure.

2.1.1. 2-Fluoro-1-[7-hydroxy-6-(4-phenyl-piperidin-1-yl)-octahydro-benzo[1,4]oxazin-4-yl]-ethanone

A two-phase-system consisting of a solution of the amine **5** (160 mg, 505 μmol) in CH_2Cl_2 (4 ml) and a mixture of $NaHCO_3$ (0.42 g, 5.0 mmol) in H_2O (3 ml) was stirred at 0–2°C. A solution of fluoroacetylchloride (37 μl, 50 mg, 518 μmol) in CH_2Cl_2 (1.0 ml) was added within 20 min. The cooling bath was removed, and after being stirred for 3 h, the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2×3 ml). The combined organic