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PET radioligands for the vesicular transporters for monoamines and acetylcholine†

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The vesicular transporters for the monoamine and acetylcholine have been successfully targeted for the development of radioligands for human brain imaging. The vesicular monoamine transporter type 2 ligands are based on the structure of tetrabenazine, a known clinically used drug. In contrast, the radioligands for vesicular acetylcholine transporter are based on vesamicol, a toxic xenobiotic. The similarities and differences in the development of these two classes of radioligands are discussed.

Keywords: fluorine-18; vesicular; transporter; brain; carbon-11

Introduction

In the development of imaging agents for neurological studies, most of the steps in the biosynthesis, storage, transport, release, receptor binding, reuptake, and catabolism of several major neurotransmitters have now been targeted.[1] For the monoamine neurotransmitters, such as dopamine and serotonin, these efforts have been quite successful and have provided a rich arsenal of radiolabeled compounds useful for examining the changes in biochemistry occurring in a wide variety of neurological and psychiatric disorders.

A central and common step in neurotransmission is the storage and release of neurotransmitters via synaptic storage vesicles. The movement of neurotransmitter molecules (either newly synthesized or being recycled via the neuronal membrane transporters) into the lumen of the storage vesicles is accomplished by an active transport system via specific proteins termed the vesicular transporters.[2] Two of these transporters have been successfully targeted for in vivo radiotracer development: the vesicular monoamine transporter type 2 (VMAT2) and the vesicular acetylcholine transporter (VAChT).[3] Although there are obligatory transporters for other neurotransmitter systems (e.g., vesicular glutamate transporter (VGLUT) for glutamate and vesicular GABA transporter (VGAT) for gamma-amino butyric acid (GABA)) no successful radiotracers have to date been developed for those amino acid vesicular transporters.

Radioligands for the vesicular monoamine transporters

The VMAT2 is a relatively unspecific transporter, as it functions to move a wide variety of amines into the vesicle lumen, including dopamine, serotonin, norepinephrine, histamine, and a number of structurally related molecules.[4,5] The VMAT2 (SLC18A1) is located primarily in the brain, although it is also found in nerves and exocrine cells in the periphery; the structurally related vesicular monoamine transporter type 1 (SLC18A2, with 40% sequence homology) is peripherally located with high concentrations in the adrenals. Vesicular transporters for the monoamines are separate from and bear no structural homology to the dopamine, serotonin, and norepinephrine neuronal membrane monoamine transporters. The VMAT2 transports positively charged amine substrates into the lumen with concomitant extrusion of two protons: the protons are generated within vesicles by the action of a separate membrane-bound adenosine triphosphatase. The amino acid sequencing of the VMAT2 has been accomplished, and the models of tertiary structure suggest a 12-transmembrane domain configuration with both the amino and carboxyl terminal ends within the cytoplasm, and a single large intraluminal loop. As the VMAT2 is not specific and is found in all monoaminergic neurons, the distinction of a neuron as being dopaminergic, serotonergic, or noradrenergic is determined by the combination of VMAT2 with the neurotransmitter-specific biosynthetic enzymes and corresponding neuronal membrane transporter. The VMAT2 is likely a necessary protein for survival in mammals, as homozygous knockout of the transporter in mice is lethal.

The successful radiotracers for in vivo imaging of the VMAT2 are all derived from the structure of tetrabenazine (TBZ: 3-isobutyl-9,10-dimethoxy-1,3,4,6,7,11b-hexahydro-pyrido[2,1-*a*]isoquinolin-2-one, Figure 1), a drug developed in the mid-20th century[6] and still in clinical use today (Xenazine®). TBZ has a high affinity for the VMAT2 (K_d values of 1–2 nM) with no appreciable affinity for any other receptor or transporter binding sites. The initial synthesis of carbon-11-labeled TBZ[7] involved the introduction of the carbon-11 into the 9-methoxy group, with the needed phenolic precursor prepared by a low yield and nonspecific demethylation using broron tribromide. Identification and assignment of the 9- versus

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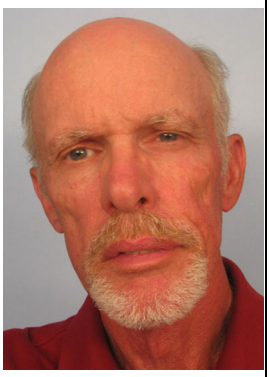
Biography



Michael R. Kilbourn was born in Detroit, Michigan, USA, in 1952. In 1980, he received his PhD in organic chemistry with John Katzenellenbogen at the University of Illinois-Urbana and then began a career in radiopharmaceutical chemistry with a postdoctoral position with Alfred Wolf at the Brookhaven National Laboratory. From there, he moved to a position as faculty member at Washington University-St. Louis, working with Michael Welch on a variety of projects in radiochemistry of positron-emitting radionuclides. He has spent the last 25 years as a professor of radiology and director of positron emission tomography (PET) radiochemistry at the University of Michigan-Ann Arbor. His research interests throughout his career have spanned the entire field of PET radiochemistry, including cyclotron targetry, new radiochemical reactions, automation methods, and synthesis and evaluation of numerous new radiopharmaceuticals for in vivo imaging of biochemistry. An emphasis on evaluating vesicular neurotransmitter transporters as targets for radiotracer development has yielded the only PET radiopharmaceuticals for these transporters in monoaminergic and acetylcholinergic neurons, which have reached the stage of applications in human studies as potential biomarkers of neuronal losses in neurodegenerative diseases. He continues active investigation into PET radiopharmaceutical development, with present research interests in the distribution and function of key enzymes involved in neurological and musculoskeletal diseases.

10-demethylation products were carried out by comparisons of proton and carbon-13 NMR spectra, and ultimately by total synthesis of the 9-desmethyl compound by an independent route. Alkylation of the 9-desmethylTBZ precursor with [^{11}C]methyl iodide[8] yielded a radiotracer that, as an isotopic substitution of the clinical drug, could be rapidly introduced into clinical studies as there were no safety questions; the pharmacology and metabolism of the drug were very well known.[9,10] The demonstration of the utility of the radiotracer to image the VMAT2 in the basal ganglia of the human,[11] with pharmacokinetics appropriate for modeling to extract estimates of important binding parameters, quickly followed.

From the initial synthesis of [^{11}C]TBZ, development of VMAT2 imaging agents took a rapidly improving pathway. The recognition that the chemical species present in the bloodstream very rapidly after TBZ administration was in fact a metabolite, 2-dihydrotetrabenazine (DTBZ: 9,10-dimethoxy-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydrobenzo[*a*]quinolizin-2-ol, Figure 1), coupled with the demonstration that the metabolite also has a high affinity for the VMAT2, suggested that the brain uptake and localization of radioactivity following [^{11}C]TBZ administration also likely represented predominantly the radiolabeled metabolite. This realization led to the deliberate syntheses and evaluation in vivo of α- and β-DTBZ, the two isomers resulting from the nonstereoselective chemical reduction of the 2-ketone group of TBZ.[8,12] The use of the higher-affinity isomer, α-DTBZ, was then quickly implemented as the preferred VMAT2 radiotracer and required only the synthesis of the needed precursor α-9-*O*-desmethylDTBZ; this was prepared in an improved yield and selectivity using demethylation with sodium hydride/*N*-methylaniline/hexamethylphosphoramide as reagent. The phenolic precursor was then alkylated with [^{11}C]methyl iodide to yield the desired α-[^{11}C]DTBZ[13]: the free secondary alcohol is unreactive under the mild basic conditions used for the methylation.

The next insight into the improvement of VMAT2 radiotracers resulted from studies using chiral column chromatography to separate the isomers of DTBZ.[14] TBZ, the clinically used drug, was and still is today utilized as a racemic mixture of stereoisomers: with two chiral carbon centers, there would be four possible stereoisomers, and the drug composition was unknown. Reduction of the ketone of TBZ produces an additional chiral carbon, resulting in eight possible stereoisomers for DTBZ. The isomers at the 2-position (termed α- and β-) are readily separated by simple column chromatography, and subsequent chiral HPLC analysis of pure α-DTBZ indicated the presence of only two stereoisomers. Careful chromatography yielded pure samples of both, from which it could be determined that the (+)-isomer (nomenclature reflecting the direction of rotation of polarized light) bound with a high affinity (approximately 1 nM), with the (−)-isomer showed negligible binding (>4000 nM).[13] The

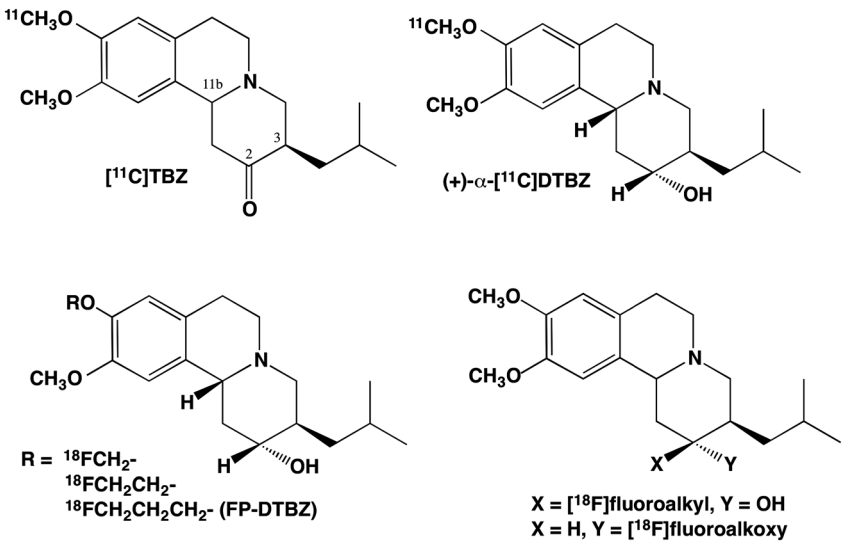


Figure 1. Positron emission tomography radioligands for the vesicular monoamine transporter type 2.

absolute stereochemistry of these isomers was next determined by X-ray crystallography of the inactive (−)-isomer, allowing an assignment of (+)-α-DTBZ as having the (2*R*,3*R*,11b*R*) stereochemistry.[14] This was the first definition of stereochemistry of DTBZ molecules: in subsequent years, all of the possible stereoisomers of DTBZ have been independently synthesized and the absolute stereochemistry and affinity for VMAT2 determined for each one.[15–17] As it turns out, (+)-α-DTBZ ((2*R*,3*R*,11b*R*) stereochemistry) is the highest affinity VMAT2 ligand amongst the eight isomers. These results also suggest the [^{11}C]TBZ previously prepared, as well as clinically used TBZ, are a mixture of the 3*R*,11b*R* and 3*S*,11b*S* enantiomers.

The syntheses of both (+)- and (−)-α-[^{11}C]DTBZ were next accomplished by the preparation of the necessary (+)- and (−)-α-9-*O*-desmethylDTBZ precursors, which could then be readily alkylated with no-carrier-added [^{11}C]methyl iodide.[18] Syntheses of the two chirally resolved desmethyl precursors were originally carried out using selective O-demethylation of racemic α-DTBZ and tedious isolation of the desired mono-demethylated product, followed by preparative chiral chromatographic separation, and isolation of the (+) and (−)-α-9-*O*-desmethylDTBZ isomers.[14] In more recent years, there have been a number of new synthetic approaches to the synthesis of TBZ and DTBZ, which allow simpler preparations of this class of molecules.[19–21]

The (+)-α-[^{11}C]DTBZ (Figure 1) has become the preferred in vivo positron emission tomography (PET) radioligand for studies of the VMAT2 in normal and diseased brain: the synthesis of the radioligand has now been reproduced by multiple research groups using solution chemistry, loop chemistry, and solid-phase-supported chemical synthesis techniques. The dynamic PET data obtained from the human PET studies can be readily applied to pharmacokinetic models, or utilized in graphical analyses, to yield estimates of binding potential for this radiopharmaceutical.[22] Studies of the VMAT2 using [^{11}C]DTBZ have now been reported in Parkinson's disease, Alzheimer's disease, Huntington's disease, schizophrenia, drug abuse, bipolar disorder, Tourettes syndrome, and other neurological and psychiatric disorders.[23,24]

The carbon-11-labeled radiopharmaceutical has proven itself a very useful compound for medical research studies. Transition of any carbon-11 compound to truly widespread clinical applications has always presented problems; this has usually been addressed by the development of analogous fluorine-18-labeled radiotracers. In the VMAT2 area, this has also been successfully accomplished. Several different approaches were taken to developing fluorine-18-labeled derivatives of (2*R*,3*R*,11b*R*)DTBZ (Figure 1), leading to potential radioligands with fluorine-18 containing substituents at the 9-position (fluoroalkyl ethers[25,26]) or at the 2-position (alkyl substitutents on the 2-carbon or ethers of the hydroxyl group[27]). The most thoroughly studied compound has been 9-*O*-(3-[^{18}F]fluoropropyl)-(+)-α-DTBZ (termed FP-DTBZ, or AV-133; 9-methoxy-10-(3-[^{18}F]fluoropropyl)-3-(2-methylpropyl)-1,3,4,6,7,11b-hexahydrobenzo[*a*]quinolizin-2-ol), prepared by [^{18}F]fluoride ion displacement of a suitably positioned tosylate or mesylate leaving group.[26,28] This simple substitution of a fluoropropyl group for the methoxy group of (+)-α-DTBZ actually resulted in a derivative with a higher in vitro binding affinity ($K_i = 0.1$ nM).[29] In vivo studies of the fluorinated derivative in rodents[26] and monkeys[30] demonstrated excellent specific localization in monoaminergic neurons of the brain, suitable pharmacokinetics, and no significant interfering metabolites. As a new chemical entity, it of course required completion of dosimetry[31] and toxicology studies prior to submission for regulatory approval for human studies. Of the numerous fluorinated DTBZ derivatives, most of which exhibit high affinity for the VMAT2, (+)-α-[^{18}F]FP-DTBZ (AV-133) remains the only radiotracer that has been applied to human clinical studies of the VMAT2 including studies in Parkinson's disease.[32–34]

Radioligands for the vesicular acetylcholine transporter

The VAChT (SLC18A3) is responsible for the movement of newly synthesized acetylcholine from the cytosol to the vesicular lumen.[3] In contrast to the VMAT2, there are no subtypes, and the VAChT is generally not found in other neuronal types than cholinergic neurons. The VAChT shares a gene locus with the acetylcholine-synthesizing enzyme choline acetyltransferase, in what has been termed the cholinergic locus. The VAChT shares some (approximately 40%) sequence homology with the vesicular monoamine transporter type 1 and VMAT2 and has been proposed to have a similar 12-transmembrane domain tertiary structure with cytoplasmic N- and C-terminal domains and a single large intra-vesicular loop. Similar to the VMAT2, the stoichiometry of exchange of acetylcholine for protons is also suggested to be 1 : 2. The structure and function of the VAChT remains, however, less fully investigated than the related VMAT2. As with the VMAT2, the VAChT is required for survival, as homozygous VAChT knockout mice die shortly after birth.[35]

From a radiotracer development point, the VAChT has proved much more difficult to approach; there are no clinically approved drugs that bind to this transporter, and in fact, inhibition of the VAChT produces severe pharmacological reactions leading to death. The development of radiotracers, therefore, has depended on radiolabeling of xenobiotics, primarily based on a compound known as vesamicol (*trans*-2-(4-phenyl-1-piperidyl) cyclohexan-1-ol, Figure 2). Originally reported in 1983, vesamicol has two chiral centers: high affinity for the VAChT ($K_i = 1–2$ nM) is observed only for the (−)-isomer (absolute configuration 1*R*,2*R*) and 25-fold lower affinity for the (+)-isomer.[36] A large number of derivatives of vesamicol have been synthesized and many show high to extremely high (subnanomolar) affinity.[36] Unfortunately, vesamicol also exhibits high binding to one or both of the sigma receptor binding sites,[37,38] and thus, early studies of radiolabeled vesamicol and simple derivatives of that structure[39] did not find successful applications for in vivo imaging.

Despite the challenges in working with the VAChT, a significant number of entirely new compounds were synthesized and many radiolabeled in the search for a useful PET radioligand for human imaging. These efforts have yielded vesamicol derivatives with added aromatic or morpholino rings, additional ring nitrogens, spirocyclic structures, or combinations of these structural changes in the attempt to develop radioligands with high affinity for the VAChT and selective against other binding sites, particularly sigma receptors.

Benzovesamicols and related as vesicular acetylcholine transporter ligands

(-)-Benzovesamicols ((-)-3-(4-phenyl-piperidin-1-yl)-1,2,3,4-tetrahydro-naphthalen-2-ol, Figure 2) have a benzene ring fused to the structure of vesamicol and in general exhibit high affinity for the VAChT. That additional aryl ring offered several positions for attachment of radiohalogens or substituents containing

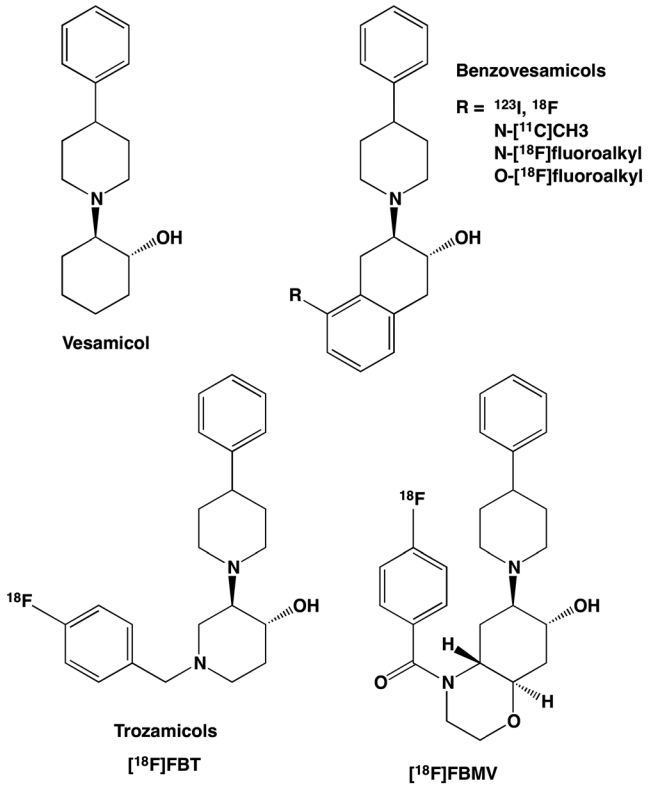


Figure 2. Positron emission tomography radioligands for the vesicular acetylcholine transporter.

carbon-11 or fluorine-18. Of the possible derivatives, it was determined that substituents at the 5-position were not detrimental and sometimes increased binding affinity, but more importantly, the 5-substituted (−)-benzovesamicols showed low or negligible affinity for sigma receptors or any other binding sites. This led to the synthesis of the earliest successful radioligand (−)-5-[^{123}I]iodobenzovesamicol, introduced into human studies in 1994[40] and in still in use at the current time.[41]

The search for a suitable PET radioligand for the VAChT based on the (−)-benzovesamicol structure[42] was initiated simultaneously with the development of a single-photon emission computed tomography radiopharmaceutical but has taken much longer to reach conclusion. Numerous carbon-11-labeled and fluorine-18-labeled 5-substituted (-)-benzovesamicols were synthesized and evaluated in animals,[42–45] as well as derivatives labeled at the opposite end of the molecule by the insertion of a ketone group into the phenylpiperidine structure.[46] Many of these derivatives showed very good brain uptake and specific localization in cholinergic structures, and several exhibit acceptable selectivity over binding to sigma receptor sites. Of the numerous compounds tested in animals, the derivative (−)-5-(2-[^{18}F]fluoroethoxy)benzovesamicol ($K_i = 1$ nM) was selected for further development into a radiopharmaceutical for human applications.[45] This radiopharmaceutical is prepared by no-carrier-added [^{18}F]fluoride ion displacement of the tosylate precursor; because of the known toxicity of VAChT inhibitors, this synthesis requires not only strict quality control measures to verify both the high specific activity needed (typically >222 TBq/mmol) but also the complete removal of the precursor and any side products (e.g., hydroxyl derivative). As this was a new chemical entity, and given the known toxicity of vesamicol derivatives, extensive preclinical animal studies including toxicology/pathology and dosimetry were completed before achieving approval for human studies. This approval has only recently been granted, but preliminary human imaging studies of the VAChT in the brain have been highly promising (K Frey *et al.*, personal communication).

In a related attempt to modify the structure of (−)-vesamicol, Sorger *et al.*[47] reported the synthesis of derivatives with substituted morpholino rings fused onto the (−)-vesamicol structure (phenylpiperidinyl-octahydro-benzo[1,4]oxazines). This series of compounds yielded derivatives with good affinity for the VAChT and selectivity for the VAChT over the sigma receptor binding sites.

Trozamicols and related vesicular acetylcholine transporter radioligands

An alternative approach to VAChT radioligands was taken in the design and synthesis of analogs of (−)-vesamicol, which were termed trozamicols (Figure 2). These are slightly different structures that still incorporate the basic structural features of vesamicol but contain an additional nitrogen in the hydroxypiperidine ring portion.[48] This provides an additional readily accessible position for introduction of radiolabeled groups such as a [^{18}F]fluorobenzyl or [^{18}F]fluorobenzoyl moiety; an example is [^{18}F]fluorobenzyltrozamicol ($K_i = 14$ nM, Figure 2). An alternative is the attachment of an N-substituted spirocyclic piperidine ring in a series of what are termed spiro-trozamicols.[49] Numerous of these substituted trozamicol and spiro-trozamicols compounds have now been synthesized, and several have been demonstrated to be useful for in vivo imaging of the VAChT in rodent and primate brain. To date, none of the trozamicol derivatives have been successfully taken to human studies.

Conclusions

The development of in vivo imaging radioligands for the two vesicular transporters, VMAT2 and VAChT, is providing interesting contrasts. The development of VMAT2 radioligands was based on a known clinically used drug (TBZ), and that allowed rapid implementation into human studies of first the isotopically labeled drug itself, followed in a reasonable time by radiolabeled derivatives meant to improve pharmacokinetics and metabolism or to provide wider availability (fluorine-18 radiolabeling). The development of VAChT radioligands depended on the modifications of a xenobiotic, vesamicol, which was a compound with known high toxicity and poor selectivity for the transporter site. Although a considerable number of analogs and derivatives of vesamicol have been synthesized and radiolabeled, progress towards human studies was impeded by the demands for full toxicological testing, and after two decades of work, only a single compound ([^{18}F]fluoroethoxybenzovesamicol) has to date reached the endpoint of evaluation in human PET studies.

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Conflict of Interest

The author did not report any conflict of interest.

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