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Targeting Cerebral Muscarinic Acetylcholine Receptors with Radioligands for Diagnostic Nuclear Medicine Studies

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1. INTRODUCTION

Nerve cells communicate via the release of chemical messengers (neurotransmitters) which bind to a site (receptor) on another or the same cell causing an effect. The various neuroreceptor classes are responsible for important functions such as movement, memory, and learning. Naturally occurring neurotransmitters are agonists, molecules that trigger an effect in the target cell after binding to the receptor site. In most cases, the binding period of an agonist to the receptor is short and after release from the receptor, the agonist is rapidly taken up by the same nerve cell (reuptake) or metabolized by various bioenzymes. Antagonists are artificial "false" neurotransmitters that bind to a receptor, often with similar or higher affinity compared to an agonist, but do not cause an effect in the target receptor other than preventing the binding of the agonist. Typically, antagonists are designed to increase the binding affinity to the receptor site, to delay release from the receptor pocket, and inhibit the metabolism by bioenzymes.

The investigation of a receptor complex utilizing a false neurotransmitter labeled with an appropriate radio isotope has allowed nuclear medicine the opportunity to image noninvasively various receptor complexes in vivo. There currently exist two imaging modalities routinely utilized in nuclear medicine studies; single photon emission computed tomography (SPECT) and positron emission tomography (PET). Radionuclides utilized in SPECT decay via the emission of relatively low energy gamma ray (~160 KeV) and

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the distribution of radioactivity is monitored utilizing an external detector. SPECT suffers from the relatively long time required to obtain sufficient counts for image processing in addition to contributions from regions that are not of interest owing to the planar-imaging aspect inherent in the technique. In contrast, PET radionuclides decay with the emission of a low-energy, positively charged beta particle (positron) that subsequently collides with an electron, resulting in annihilation of the electron with a release of two 511 KeV gamma rays emitted at 180° with respect to each other. An external ring of detectors detects this dual emission and allows the instantaneous processing of the event, affording a higher quality image with greater resolution in a shorter acquisition time. This attractive property allows for the modeling of kinetic binding parameters of the ligand-receptor interaction for a better understanding of receptor viability in healthy and diseased tissues. Advances in SPECT hardware and software technology has resulted in the development of two-head, three-head, and ring systems with increased sensitivity and resolution, affording imaging time, quality, and resolution similar to that obtained with PET instrumentation.

This chapter will discuss radiolabeled antagonists that demonstrate selective binding to the muscarinic acetylcholinergic receptor (mAChR) in preclinical and clinical trials for the noninvasive in vivo imaging of cerebral mAChR. In addition, the development of subtype-selective antagonists for the individual evaluation of the various mAChR subtypes will be addressed.

2. MUSCARINIC RECEPTOR COMPLEX

Many properties of mAChR have been known since ancient times and the physiological neurotransmitter signal molecule, acetylcholine—first observed as a contaminant in various biological preparations—was identified in 1914. This was followed by the discovery of an enzyme, cholinesterase, which degrades acetylcholine in vivo *(1)*. The mAChR protein consists of seven putative membrane-spanning regions that are characteristic of the super family of G-protein-coupled receptors. They are incorporated into the sarcolemma, protruding into both the extra and intracellular spaces with the amino terminus of the peptide located outside the cell and the carboxyl terminus localized inside the cell.

An important advancement in the study of mAChR was the development of subtype-selective ligands in the late 1970s that allowed for the identification of four subtypes, designated M_1–M_4, by classical pharmacological methods *(2)*. Recently, five isoforms (m1–m5) were identified utilizing molecular cloning technique *(3)*. Subsequent studies have shown the cor-

relation between the pharmacologically and genetically defined subtypes: M_1 = m1, m4, m5; M_2 = m2; M_3 = m3. (In this chapter, the pharmacological receptor subtypes will be designated as M_1 and the cloned subtypes designated as m1.) The m1, m3, and m5 subtypes preferentially activate phospholipase C via a pertussis toxin insensitive G-protein ($G_{q/11}$ family of proteins) catalyzing phosphoinositide breakdown and the m2 and m4 subtypes inhibit adenyl cyclase activity via a different pertussis toxin-sensitive G-protein ($G_{i/o}$ family of proteins) *(4)*. Receptor desensitization via phosphorylation is a reversible process, can occur within seconds, and involves phosphorylation of the receptor and its associated G protein.

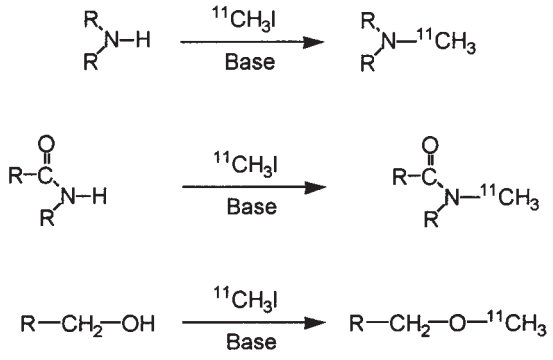
Cerebral M_1 binding sites are localized postsynaptically and M_2 sites are predominately located presynaptically. The mAChR subtypes are located in various concentrations in cortical and subcortical brain regions *(5)*. The m1 subtype is most abundant in the cortex (40%), striatum (31%), and hippocampus (35–60%). Lower levels of the m1 subtype are reported in the nucleus basalis (15–20%). The m2 subtype is enriched in the occipital cortex (36%), nucleus basalis (41%), thalamus (50%), cerebellum (88%), and pons/medulla (81%). The m4 subtype is enriched in the putamen (50%), with lower amounts in cortical regions, hippocampus, and nucleus basalis (13–23%). The m3 and m5 subtype proteins have been detected at significantly lower levels in these cerebral regions.

Change in density or function of cerebral mAChR have been implicated in aging, sudden infant death syndrome (SIDS), memory, sleep disorders, alcoholism, Parkinson's disease (PD), and various dementias such as Alzheimer's disease (AD) or *(6–11)*. This has spurred interest in the development of radiolabeled false neurotransmitters for imaging cerebral mAChR to aid in a greater understanding of the role of mAChR in normal and disease processes.

3. LABELING CHEMISTRY

Fluorine-18 (F-18, $t_{1/2}$ = 110 min) and carbon-11 (C-11, $t_{1/2}$ = 20 min) are two widely utilized radio isotopes for labeling PET neurotransmitters, owing to their favorable decay properties, and are cyclotron produced in high specific activity (to stable isotopes). However, their short half-lives often preclude distribution to other sites and necessitates the need to produce these isotopes onsite increasing the cost for their use.

The development of SPECT ligands is important owing to the large number of medical centers routinely using SPECT systems, allowing this technology to benefit a larger patient population at a lower cost. Iodine-123



Scheme 1. Incorporation of C-11 into an amide or ether position.

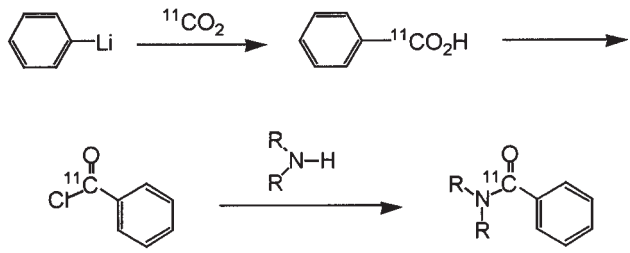
(I-123) is well suited for SPECT owing to its half-life (13.2 h) and imagable gamma ray (158.9 KeV). The longer half-life is advantageous because radiopharmaceuticals can be prepared in a regional pharmacy and shipped to various nuclear medicine centers in a large area, thereby lowering the cost and increasing the availability. In addition, there are two iodine radio isotopes, I-125 and I-131, which are commercially available at low cost with suitable specific activity for the initial development of new radioiodinated ligands.

3.1. Carbon-11

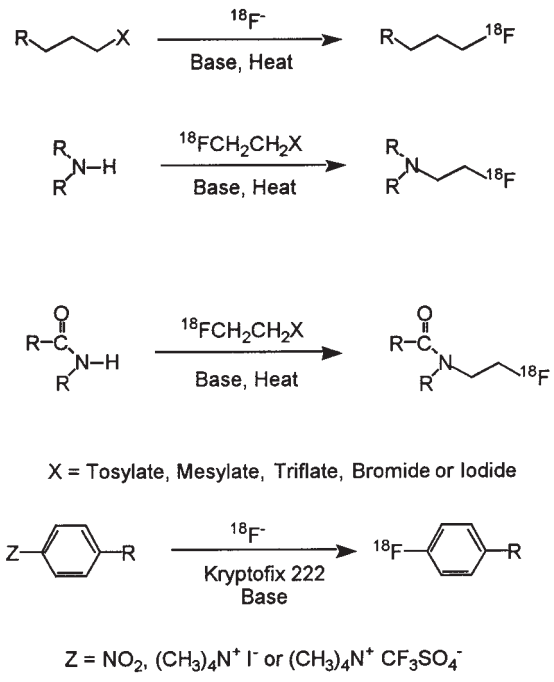
The incorporation of C-11 is routinely investigated because many neurotransmitters contain a functional group that C-11 can be readily incorporated into under mild conditions. Owing to the short half-life, many facile methods have been optimized for rapid C-11 incorporation (Scheme 1). A widely utilized method is the addition of a labeled methyl group via C-11 labeled methyl iodide or methyl triflate to a secondary nitrogen or hydroxyl moiety to afford a tertiary amine, amide, or ether, respectively *(12)*. The introduction of the radiolabeled group at the final stage of synthesis affords a purified radiopharmaceutical with high specific activity. Another routinely utilized method is the introduction of the C-11 into a carbonyl moiety via C-11 labeled carbon dioxide (Scheme 2) *(13)*. However, in many cases, the C-11 label is introduced earlier in the synthesis, resulting in a longer synthesis time and affording a lower radiochemical yield and specific activity owing to the decay of the C-11.

3.2. Fluorine-18

The introduction of F-18 is not as straightforward because naturally occurring neurotransmitters do not contain a fluorine atom. However the

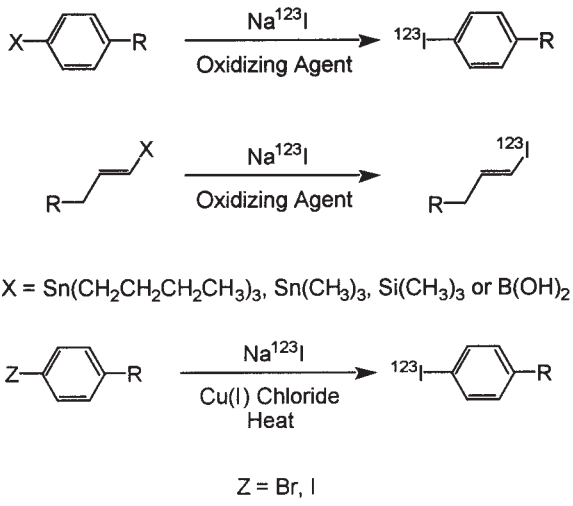


Scheme 2. Incorporation of C-11 into a carbonyl moiety.



Scheme 3. Incorporation of F-18 into an alkyl, amine, amide, or aromatic position.

longer half-life allows for F-18 incorporation at an earlier stage in the synthesis and more elegant chemistry can be utilized. F-18 is usually introduced as a fluoroalkyl group or placed in a biologically inactive position on a phenyl ring via a nucleophilic displacement of a suitable leaving group (Scheme 3) *(14,15)*.



Scheme 4. Incorporation of radioiodine into an alkenyl or aromatic moiety.

3.3. Iodine-123

Analogous to F-18, naturally occurring neurotransmitters do not contain an iodine atom. The introduction of an iodine moiety can cause a dramatic increase in the lipophilicity hindering the ability of the ligand to effectively cross the blood-brain barrier (BBB) and thus lowering the level of tracer available for binding. In addition, the relatively large size of iodine has the potential to interfere in the receptor-binding pocket interactions. Iodine is typically placed in a metabolically stable position on an aromatic ring or an alkenyl group via electrophilic iododemetallation of an alkyl stannyl, silyl, or boronic intermediate with sodium iodine (Scheme 4) *(16)*. An alternative method for iodine incorporation is via a halogen exchange reaction *(17)*. However, a decrease in specific activity of the radiopharmaceutical is realized when iodine for iodine exchange is utilized and may hinder its use as a suitable tracer owing to competition of the unlabeled ligand for the receptor site.

4. SPECT IMAGING STUDIES

4.1. R,S-IQNB

R-1-Azabicyclo[2.2.2]oct-3-yl α-hydroxy-α,α-phenylacetate (QNB) has been shown to be a potent mAChR antagonist, and a proposed "three points of attachment" model for binding of QNB to the receptor did not explain the role of the second phenyl ring *(18,19)*. The addition of an iodine atom to one

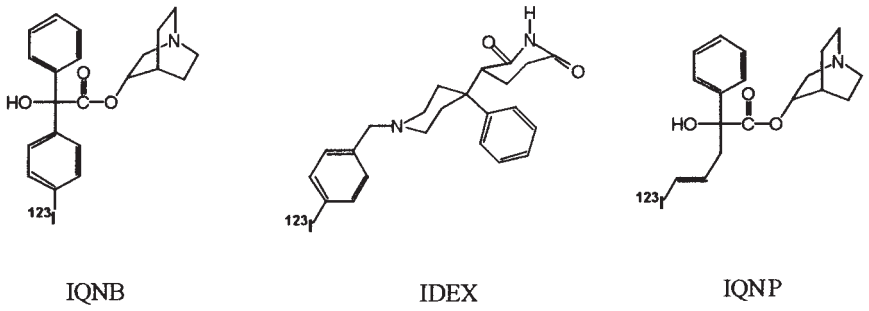


Fig. 1. Radioiodinated ligands developed for SPECT studies of mAChR.

of the phenyl rings was observed to afford an analog, 3-quinuclidinyl 4-iodobenzilate, (IQNB, Fig. 1), which retained a high mAChR binding affinity in vitro *(20)*. This modification inferred chirality (i.e., absolute asymmetry in space) to the benzilic center and in addition to the chirality of the quinuclidinyl ring, racemic IQNB contains four stereoisomers. Upon separation (resolution) of the stereoisomers, R,S-IQNB was observed to be slightly m1 selective (12-fold) in vitro but did not demonstrate subtype selectivity in vivo *(21)*. (Throughout this chapter, the first stereochemical designation refers to the heterocycle ring and the second stereochemical designation refers to the benzilic moiety.) In initial animal studies, a positive correlation between the mAChR concentration and R,S-IQNB binding was demonstrated in various cerebral structures *(22)*.

R,S-IQNB was subsequently labeled with I-123 and the first in vivo imaging of cerebral mAChR in a healthy volunteer was reported and demonstrated the potential utility of nuclear medicine technology for the imaging of mAChR *(23)*. Subsequent studies followed in healthy individuals and patients with dementias *(24–29)*. These studies demonstrated a measured difference in the distribution of activity between IQNB binding and blood flow as measured by technetium-99m (Tc-99m) HMPAO, an established blood flow tracer. Another study compared regional glucose metabolism utilizing F-18-2-deoxyglucose (FDG) to IQNB binding and larger defects were observed in IQNB binding as compared to FDG utilization in patients diagnosed with dementia, although a better understanding of receptor quantification was required to determine various ligand-binding parameters.

Although in vitro binding assays indicate R,S-IQNB not to be subtype selective, owing to the delayed time between radiotracer administration and imaging required to allow sufficient localization of radiotracer and clearance of nonspecific uptake (approx 24 h), R,S-IQNB accumulated in cere-

bral regions containing a high concentration of the M_1 (m1, m4) subtype and does not allow for the imaging of the M_2 (m2) mAChR subtype.

4.2. Iododexetimide

The successful in vivo imaging of mAChR utilizing R,S-IQNB spurred the development of new radiopharmaceuticals to improve imaging properties, such as an increased ability to pass the BBB and a rapid clearance of nonspecific binding. Dexetimide, another established potent muscarinic antagonist, was reported to accumulate in cerebral regions containing a high concentration of mAChR in a stereospecific and saturable manner *(30)*. When labeled with I-123, dexetimide (IDEX, Fig. 1) retained a high specificity for cerebral mAChR in vitro and in vivo although not demonstrating mAChR subtype selectivity *(31)*.

In studies involving healthy individuals, IDEX showed favorable dosimetry, high specific binding, and a clear binding profile not related to blood flow, affording high quality images reflecting the known distribution of mAChR at 6 h postinjection *(32,33)*. As observed for R,S-IQNB, the activity accumulated in the cortex and striatum with minimal uptake in cerebellum and thalamus, indicating an apparent in vivo selectivity for M_1 rich cerebral regions.

IDEX has been utilized in studies of patients with temporal lobe epilepsy *(34–36)* and although these studies show IDEX binding to be decreased in the hippocampal region, no difference in IDEX binding was detected when a partial volume correction was applied to the image analysis. In a study involving patients diagnosed with mild AD, IDEX binding was observed to be reduced in patients with mild probable AD in the temporal and temporoparietal cortex as compared to controls *(37)*.

4.3. IQNP

Recently, we evaluated a novel QNB analog, 1-azabicyclo[2.2.2]oct-3-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-phenylacetate (IQNP, Fig. 1), in which the modification of one of the phenyl rings of QNB to an iodopropenyl moiety afforded a ligand with decreased lipophilicity and the potential for facile passage across the BBB *(38)*. Owing to the presence of two asymmetric centers and the *cis-trans* isomerization of the double bond, racemic IQNP contains eight stereoisomers. Upon resolution of the stereoisomers, E-R,R-IQNP was observed to have an 80-fold higher binding affinity for the M_1 (m1, m4) subtype over the M_2 (m2) subtype and Z-R,R-IQNP demonstrated a high nonsubtype selective binding affinity in vitro *(39)*.

Subsequent in vivo rat biodistribution studies utilizing E- and Z-R,R-IQNP supported this significant difference in binding to the various mAChR

subtypes *(40,41)*. In vivo autoradiographic studies in rats showed that although both the E- and Z-R,R-isomers label the thalamic nuclei to the same degree, the E isomer labeled the pons, colliculis, and cerebellum to a lesser degree. An ex vivo autoradiographic study utilizing healthy human brain slices obtained at autopsy showed pretreatment with biperiden (M_1 selective antagonist) inhibited the binding of E-R,R-IQNP *(42)*. Subsequent nonhuman primate imaging studies have also confirmed a significant difference in E- and Z-R,R-IQNP binding to the M_1 and M_2 subtypes *(42–44)*.

A unique feature of iodine in the development of new radiopharmaceuticals is the availability of three radio isotopes with sufficient specific activity that can be employed in a single in vivo biological study. By individually analyzing the decay energy and half-life of each isotope, a direct in vivo comparison of different ligands or stereoisomers is possible, allowing each animal as its own control. In this manner the effects of age, injection technique, circadian rhythm, and diet; for example, do not distort the data and absolute differences in receptor binding can be evaluated. In a dual-label study, I-125-Z-R,R-IQNP and I-131-E-R,R-IQNP were administered to the same animal and, in agreement with previous individual studies, E-R,R-IQNP bound selectively to cerebral regions containing a high concentration of the M_1 (m1, m4) subtype and Z-R,R-IQNP demonstrated nonselective binding in vivo at 6 h postinjection *(41)*. A triple-label study was also performed utilizing I-123-Z-R,R-IQNP, I-131-IDEX, and I-125-R,S-IQNB and Z-R,R-IQNP was observed to accumulate to a higher degree in all mAChR regions. More importantly, Z-R,R-IQNP is the first example of an iodinated ligand demonstrating a significant and prolonged binding in cerebral regions containing a high concentration of the M_2 subtype. Initial SPECT studies in progress with I-123-E- and Z-R,R-IQNP in healthy volunteers have also confirmed the difference in subtype mAChR binding of these isomers (Fig. 2) *(45)*.

5. PET IMAGING STUDIES

5.1. Scopolamine

Because C-11 can be conveniently incorporated directly without a compromise in the biological behavior of the ligand, scopolamine (SCOP, Fig. 3), an established mAChR antagonist, was first investigated for imaging mAChR via PET *(46)*. Preliminary in vivo studies in rats suggested the cerebral distribution of radioactivity paralleled mAChR populations and a subsequent PET study performed in six healthy individuals demonstrated the potential for imaging mAChR via PET *(47)*. However, it was concluded the

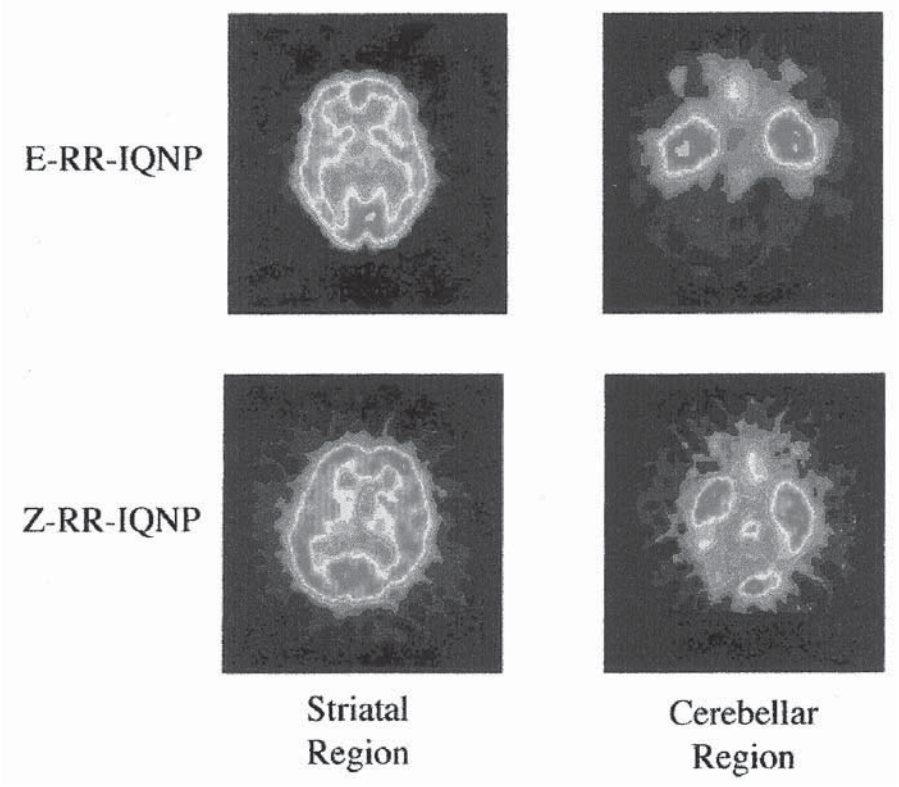


Fig. 2. Initial human volunteer SPECT imaging study utilizing I-123-E- and Z-RR-IQNP (100 MBq) at 30 h postinjection. (Used with permission of Nobuhara, Halldin et al., Karolinska Institute, Stockholm, Sweden.)

use of SCOP was not an ideal tracer owing an unfavorable binding profile and lack of an appropriate compartmental kinetic binding model arising from the short half-life of C-11.

5.2. QNB, Dexetimide, Benzotropine

The promising results of SCOP lead to the subsequent C-11 labeling of QNB, dexetimide (DEX), and benztropine (BENZ) (Fig. 3) *(48–50)*. Of these ligands only C-11 BENZ has been utilized for the evaluation of mAChR in the human brain and the localization of radioactivity was shown to be specific for M_1 mAChR rich cerebral areas at 60 min postinjection *(50)*. In a study evaluating age-related changes in mAChR binding, a decrease in BENZ binding was observed in regions of high density as com-

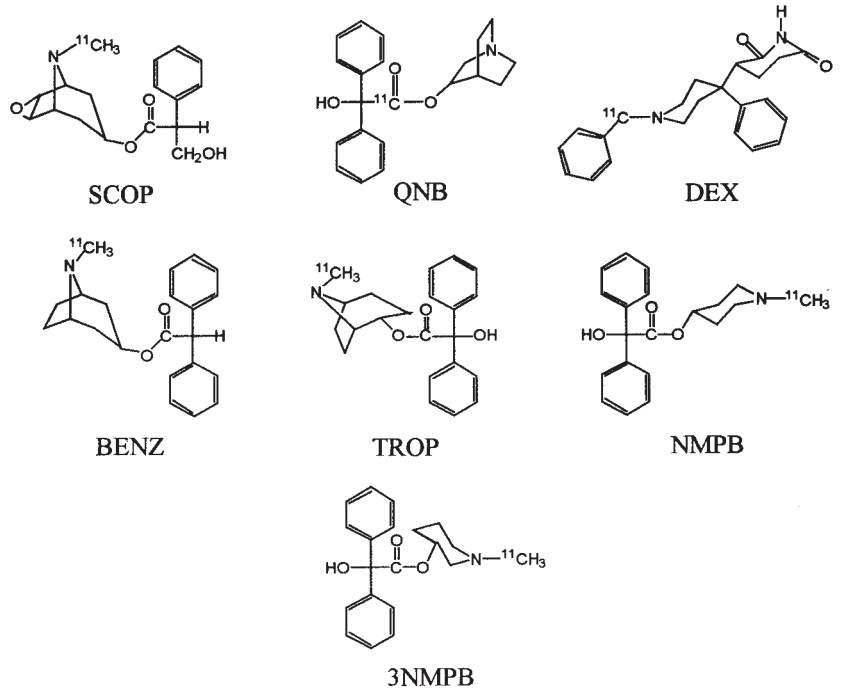


Fig. 3. C-11-labeled ligands developed for PET studies.

pared to regions containing a relatively low density *(51)*. However, as reported for SCOP, owing to the short half-life of C-11, the prolonged time required for clearance of nonspecific binding to allow a better delineation of receptor binding in receptor-rich areas was not achieved. As techniques for processing PET data have improved overcoming the limited study time of C-11 and the resultant poor receptor binding kinetics, a study involving six healthy volunteers reported the anatomical standardization of cerebral mAChR images obtained utilizing BENZ have the potential to delineate physiological or pathological alterations *(52)*.

5.3. Tropinyl Benzilate

To overcome long equilibration times as compared to transport rates that make it difficult to evaluate mAChR viability accurately, C-11 tropanyl benzilate (TROP, Fig. 3), was investigated *(53)*. Imaging studies conducted in female pigtail monkeys demonstrated at 50–80 min postinjection regional localization of activity was consistent with mAChR distribution. Subsequent imaging and kinetic analysis in six young, healthy volunteers demonstrated

reliable receptor density information and an appropriate compartmental kinetic model could be obtained *(54)*. A subsequent study to quantify mAChR in normal aging was performed and, as opposed to other studies and clinical evidence, cerebral cortical cholinergic dysfunction in elderly subjects was not attributable to a major loss of total muscarinic cholinoceptive capacity *(55)*.

5.4. 4-N-Methylpiperidinyl Benzilate

A decrease in the ligand binding affinity may afford improved ligand-receptor binding profile required for the development of kinetic compartmental model for the determination of receptor function and viability. 4-*N*-Methylpiperidinyl benzilate (NMPB, Fig. 3), the simplest of a series of N-substituted benzilic esters, displays a lower binding affinity as compared to QNB *(56)*. An initial PET study utilizing C-11-NMPB allowed the development of an appropriate compartmental model for the quantification of mAChR and an accurate distinction of receptor binding parameter *(K_3)* to estimate tracer delivery with acceptable precision in both intra- and intersubject comparison *(57)*.

In an aging study, a 45% age-related decrease in K_3 was observed over the age range of 18–75 yr *(58)*. In another study, patients were evaluated for mAChR occupancy after administration of trihexyphenidyl (anticholinergic drug), L-dopa (antiparkinson drug), or triazolam (insomnia drug) to evaluate the effect of drug therapy in the treatment of various dementias *(59,60)*. It was observed the administration of a therapeutic dose of trihexyphenidyl caused a mean 28% inhibition of K_3 and after administration of L-dopa no significant change in K_3 was observed. Administration of triazolam, reported to decrease cortical acetylcholine release, also caused a decrease in the binding of NMPB in the temporal cortex and thalamus. This study demonstrates the potential of nuclear medicine for the measurement of drug-induced changes in mAChR occupancy for more effective management of drug therapies.

In patients diagnosed with PD, K_3 values were observed to be 20% higher in the frontal cortex as compared to age-matched controls *(61)*. However, alterations in cerebral blood flow (CBF) has the potential to impart a significant influence on the results and must be taken into account when interpreting the data, for example, an increase in CBF is expected to cause an increase in ligand delivery and a higher cerebral accumulation of activity. Because a decreased or unchanged CBF has been reported in patients diagnosed with PD, the increase in NMPB binding is owing to deviations in mAChR. It was postulated the increase in NMPB binding was a response to a loss of ascending cholinergic input to the frontal cortex and relates to frontal lobe dysfunction.

In another study addressing the important role of CBF on the interpretation of radiopharmaceutical-receptor binding studies, cerebral glucose metabolism (via F-18-FDG), CBF (via Tc-99m-HMPAO), and mAChR binding (via C-11 NMPB) were measured in 18 patients diagnosed with "probable AD" *(62)*. A significant decrease in NMPB binding, a greater decrease in glucose metabolism and a slight decrease in the CBF were observed in patients diagnosed with mild or moderate AD as compared to controls. This study suggests that although there was decrease in mAChR binding, FDG was a more sensitive tool for the detection of degenerative cerebral regions in AD.

In a study evaluating of the role of mAChR in human narcolepsy, NMPB uptake in the pons, thalamus, striatum, and cerebral cortex was measured in 11 patients and 21 normal controls *(63)*. In addition, 7 of the 11 patients underwent additional PET scans after alleviation of the symptoms by drug therapy. No difference in NMPB binding between the patients and controls before or after drug therapy was observed and does not support a major role for drug therapy targeted at mAChR for the clinical improvement of human cataplexy.

5.5. (+)-N-Methyl-3-Piperidinyl Benzilate

(+)-*N*-Methyl-3-piperidinyl benzilate (3NMPB, Fig. 3), an analog of NMPB, displays a decreased in vitro binding affinity as compared NMPB and may afford improved binding kinetic parameters for quantification of mAChR function *(64)*. Utilizing in vitro autoradiographic analysis in rats, 3NMPB exhibited high accumulation in the corpus striatum, hippocampus, colliculis, and cerebral cortex, but the issue of receptor binding equilibrium has not yet been addressed.

5.6. Fluorine-18-Labeled Ligands

F-18, with its longer half-life, has the potential for a longer imaging protocol to allow nonspecific binding to clear and to achieve ligand-binding equilibrium for a higher target to nontarget tissue ratio. Surprisingly, only the initial evaluation of fluorodexetimide (FDEX) *(65)*, 1-azabicyclo[2.2.2]oct-3-yl α-(1-fluoropentan-5-yl)-α-hydroxy-α-phenylacetate (FQNPe) *(66)* and *N*-fluoroethyl-4-piperidinyl benzilate (FNEPB) have been reported (Fig. 4) *(67)*.

6. DEVELOPMENT OF mAChR SUBTYPE-SPECIFIC LIGANDS

The radiopharmaceuticals discussed earlier demonstrate the potential for the noninvasive in vivo evaluation of mAChR function and viability utiliz-

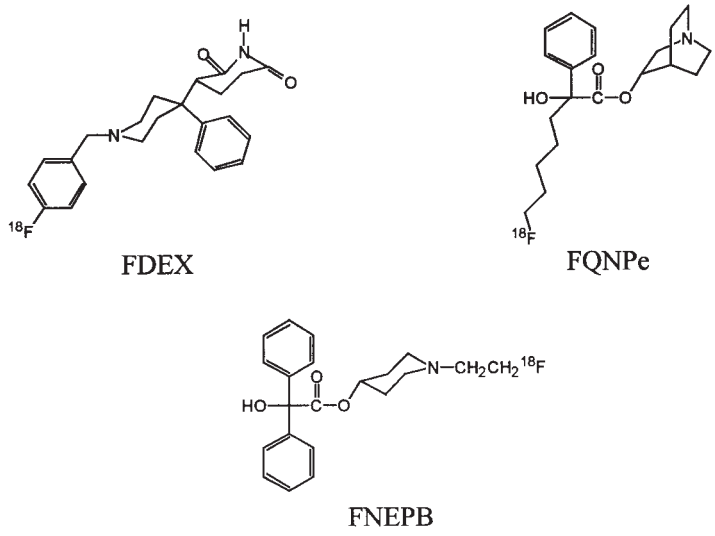


Fig. 4. F-18-labeled ligands developed for PET studies.

ing nuclear medicine protocols. However, a major disadvantage is the lack of demonstrated mAChR subtype selectivity of these radiopharmaceuticals. The ability to image the various mAChR subtypes independently is of importance owing to the role the mAChR subtypes are postulated to play in various biological processes and diseases. For example, the m2 subtype is postulated to decrease in the early stages and, as a response, the m1 subtype is postulated to be upregulated in later stages of AD. The ability to image selectively the m2 mAChR subtype is therefore of importance for an early diagnosis and a better understanding of the progression of AD. Another expected benefit for the selective subtype imaging of mAChR is the monitoring of various drug regiments to afford more effective treatments. Therefore, there is a need for the design and evaluation of new radiopharmaceuticals targeted to the individual mAChR subtypes.

6.1. PET Ligands

5,11-Dihyro-8-chloro-11-[4-[3-[(2,2-dimethyl-1-oxopentyl)ethylamino]-propyl]–1-piperidinyl]-acetyl]–6H-pyrido[2,3-b][1,4]benzodiazepin-6-one (BIBN 99, Fig. 5) is a lipophilic M_2 antagonist with central nervous system (CNS) activity, able to cross the BBB, and demonstrates a m2/m1 selectivity of 33 to 1 in vitro *(68)*. C-11-BIBN 99 has recently been reported although the in vivo biological evaluation was not discussed *(69)*.

A fluorinated QNB analog, R-1-azabicyclo[2.2.2]oct-3-yl S-α-hydroxy-α-(4-fluoromethyl)phenyl-α-phenylacetate (FMeQNB, Fig. 5), was shown to have a m2/m1 selectivity of 7 to 1 in vitro *(70)*. In rats studies, F-18-FMeQNB uptake was nearly uniform in all brain regions, reflecting the reported m2 subtype concentration, and was reduced by 36–54% in all cerebral regions following co-injection of unlabeled FMeQNB. In subsequent monkey PET imaging studies, FMeQNB demonstrated cerebral uptake with slow clearance that was displaced by QNB to a greater extent in the thalamus (high m2 concentration) as compared to the cortex (low m2 concentration). These results support the assumption that FMeQNB displays selective m2 binding in vivo but until the development of a proven m2 subtype selective ligand able to cross the BBB, the subtype selectivity of FMeQNB cannot be firmly established. As observed for other QNB analogs, FMeQNB demonstrates a slow receptor binding off-rate that prohibits the determination of various kinetic parameters required for the evaluation of mAChR viability.

6.2. SPECT Ligands

The modest ability of E-R,R-IQNP to differentiate between the M_1 and M_2 mAChR subtypes in vitro and in vivo is encouraging in the development of radioiodinated subtype ligands. Changes in the benzilic or the heterocycle moieties of QNB have been shown to influence mAChR binding affinity and subtype selectivity of new analogs. An IQNP analog, 1-methylpiperidin-4-yl α-hydroxy-α-(1-iodo-1-propen-3-yl)-α-phenylacetate (IPIP, Fig. 5) has been prepared in which the quinuclidinyl ring was modified to a 1-methylpiperidin-4-yl ring and resulted in a decrease in the lipophilicity allowing a more effective passage across the BBB *(71)*. The individual I-125 IPIP isomers were evaluated in vivo in rats and demonstrated a rapid and high accumulation in regions of the brain containing mAChR. Although not demonstrating an increased cerebral uptake, E-R-IPIP selectively accumulated in M_1 rich cerebral regions by 4 h postinjection. Z-S- and Z-R-IPIP initially demonstrated a significant and uniform uptake in cerebral regions containing mAChR indicating nonsubtype selectivity with Z-S-IPIP demonstrating similar kinetic binding as observed for Z-R,R-IQNP. Z-R-IPIP, however, demonstrated a faster release from the M_2 site and may have potential in the evaluation of changes in receptor function in M_2 rich cerebral regions.

IPIP is a member of a unique class of radiopharmaceuticals that can be radiolabeled either with I-123 on the allyl position for SPECT studies or by C-11 in the N-methyl position for PET studies. Ex vivo metabolic studies with Z-S-IPIP demonstrated the N-methyl group to be stable in vivo and the desmethyl compound does not cross the BBB. In addition, if IPIP is metabo-

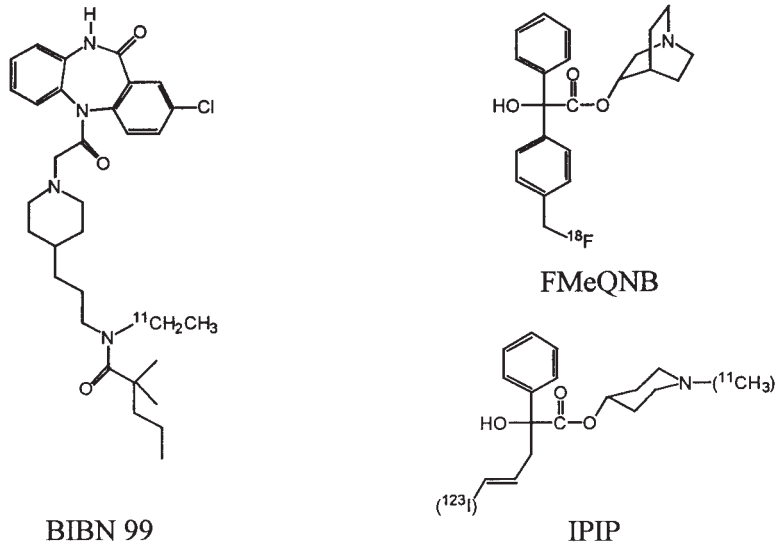


Fig. 5. Ligands investigated for evaluation of mAChR subtypes.

lized after release from the receptor pocket, potential metabolites were not observed to compete with IPIP binding at the receptor site making this position attractive for C-11 labeling. Initial studies have shown E-R-IPIP is readily radiolabeled with C-11 under mild conditions *(72)*.

7. CONCLUSIONS

The development of radiopharmaceuticals targeted to mAChR have demonstrated the potential for the detailed study of mAChR density and viability by nuclear medicine imaging techniques for a variety of biological processes and disease states. The evolution of appropriate kinetic binding models for interpretation of imaging studies have allowed a better understanding of the radiopharmaceutical-receptor binding kinetics in vivo. Although a variety of "false" neurotransmitters labeled with either C-11, F-18, or I-123 have been developed, these radiopharmaceuticals do not possess ideal receptor binding properties to allow a complete understanding of kinetic binding parameters of the ligand-receptor interaction. Future developments in radiopharmaceutical selectively design include the ability to bind the radiopharmaceutical to individual mAChR subtypes for a better understanding of the role of the mAChR subtypes in aging, memory, movement, and disease processes. Improved imaging protocols to optimize ligand-

receptor kinetic models will afford a more detailed modeling and understanding of the receptor binding parameters. In addition, this new generation of mAChR subtype selective radiopharmaceuticals will allow for an increased role of nuclear medicine studies as an accepted tool in the early diagnosis and treatment of various dementias and diseases. Nuclear medicine protocols will also serve as an important tool in the monitoring of various drug regiments affording improved drug therapy and an efficient, cost-effective modality of treatment leading to an improvement in the quality of life.

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