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Clinical Perspective and Recent Development of PET Radioligands for Imaging Cerebral Nicotinic Acetylcholine Receptors

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IMAGING NICOTINIC ACETYLCHOLINE RECEPTORS WITH PET IN HUMAN SUBJECTS

There have been a number of attempts to study the nicotinic cholinergic system in the human brain and the only successful approaches have been for the $\alpha_4\beta_2$ subtype of the nicotinic acetylcholine receptor (nAChR), the main cerebral nAChR subtype. The first and perhaps the most straightforward approach was to study the active and less active isomers of [¹¹C]nicotine [(–)-enantiomers (Fig. 1) and (+)-enantiomers, respectively].¹ These were among the earliest PET tracers to be used in human beings because of the known toxicology of nicotine and their ease of radiolabeling. Several efforts have been made to quantify [¹¹C](–)-nicotine and use the less active [¹¹C](+)-nicotine to assist in measuring nonspecific binding. Measures of perfusion with [¹⁵O]water have also been used to aid in the quantification of nAChR binding.² However, a number of investigators^{2–4} have concluded that, although specific binding could be measured, there were large changes in specific binding reflected by small changes in distribution volume and that neither the (–)- nor the (+)-[¹¹C]nicotine were very suitable radiotracers.

In subsequent years, fluorinated derivatives of A-85380 labeled in both the 2 and the 6 position of the pyridine ring have been considered as promising radiotracers for imaging $\alpha_4\beta_2$ -nAChRs (see Fig. 1).

One of the most popular, 2-[¹⁸F]FA (see Figs 1 and 2, Table 1), has been shown to have a radiation effective dose of 39.6 mSv/MBq and radiation dose-equivalent value for the critical organ urinary bladder of 461 μ Sv/MBq.¹⁹ This allows at least two or more injections using typical state-of-the-art PET cameras and setting five Rem as the effective dose for an annual occupational limit. Toxicology studies with 2-FA typically show safety margins in excess of 1,000-fold with no change in blood pressure or heart rate.¹⁹ Unlike the very toxic epibatidine derivatives, which were initially considered for [¹⁸F] radiolabeling for human use and studied

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in primates,^{20–25} 2-[¹⁸F]FA and the related derivative 6-[¹⁸F]FA have provided the field with radiopharmaceuticals for multiple brain applications that have acceptable toxicity and low radiation risk.

2-[¹⁸F]FA is not entirely ideal because it does not reach a steady state for several hours after injection, but it can be quantified by number of procedures in the human brain. Some of these have included dynamic PET scans over 2 hours followed by repeat scans an hour later. With arterial sampling and compartmental modeling, this technique has provided quantifiable measures of distribution volume that is regionally specific and stable in the brain.^{7,8} Bolus injection studies have employed a scanning time of only 140 minutes and two-tissue compartmental models but do require arterial blood sampling. Other methods, such as those employed by Brody and colleagues²⁶ and Kimes and colleagues⁹ have performed 2-[¹⁸F]FA studies using bolus-plus-infusion methods with a duration of 6 to 8 hours ($K_{\text{bolus}} = 500$ minutes). Similarly, both bolus and continuous infusion methods have been performed with the single-photon emission computed tomography nAChR radioligand 5-[¹²³I]IA.^{18,27}

A number of attempts have been made to simplify the 2-[¹⁸F]FA studies. For example, a simplified distribution volume determined by the ratio of the tissue to the metabolite corrected plasma and using a single 90- to 120-minute PET acquisition appeared to correlate reasonably well with graphical methods and two compartmental models in cortical regions. Lower correlations were found in regions with higher binding of nicotinic cholinergic receptors, such as the thalamus. This lower correlation in higher binding regions is not unexpected because the time to achieve steady-state conditions is even slower than in the lower binding regions.¹³

In addition to the extensive use 2-[¹⁸F]FA, there is increasing interest in derivatives such as 6-[¹⁸F]FA.²⁸ Based on nonhuman primate studies, some centers believe that 6-[¹⁸F]FA shows more reversible kinetics and higher specific binding than 2-[¹⁸F]FA.²⁹ The initial studies with 6-[¹⁸F]FA¹⁷ in five human subjects suggested some reversibility in various brain regions, with the radiotracer peaking at 1 to 2 hours in the thalamus. The half-life for clearance was about 4 hours. Cortical binding peaked early in less than 60 minutes, but there was lower—but persistent—uptake in white matter. Retest studies ranged from as low as 0.5% to as high as 14% in various brain regions. Relatively high toxicity of 6-FA is an obstacle for more wide use of 6-[¹⁸F]FA as a PET radioligand for human studies.³⁰

Given the success with both 2-[¹⁸F]FA and 6-[¹⁸F]FA, a number of successful applications have been performed. Among the first, not surprisingly, were studies of cigarette smokers. For example, a recent study²⁶ demonstrated that there was a dose-dependent effect of smoking from one puff, to three puffs, to a full cigarette or to satiety (around ~3 cigarettes), demonstrating a dose-dependent occupancy in nAChR-rich regions. Most importantly, it suggested that one to two puffs of the cigarette resulted in 15% occupancy for more than 3 hours after smoking. A full cigarette resulted in greater than 88% occupancy, and the investigators concluded that daily smoking results in near complete occupancy of nAChRs, suggesting that tobacco-dependent smokers maintain almost full occupancy throughout the day.

Another example of an application in patients includes 2-[¹⁸F]FA PET images of medial frontal-lobe epilepsy demonstrating a significant increase in the distribution volume in the mesencephalon, pons, and cerebellum when compared with controls. This, together with FDG measurements, suggested that the mesencephalon might be related to arousal.¹⁴

Because of the importance of nicotine and memory, and the known $\alpha_4\beta_2$ abnormalities seen in postmortem studies, 2-[¹⁸F]FA studies have been explored in Alzheimer's disease.^{11,12,15} One example^{11,12} was unable to demonstrate loss of nAChRs in vivo in early Alzheimer's disease, in spite of the fact that these patients showed significant cognitive impairment. Although this

could be related to the sensitivity of the technique, it could also illustrate the ability of the technique to separate a lack of change in spite of cognitive decline in Alzheimer's disease patients versus controls. This is in contrast to postmortem studies, where the decline was always demonstrated, but perhaps at a later stage in the disease. Other studies, such as those of patients with mild dementia and mild cognitive impairment, demonstrate a reduction of nAChRs in some subjects with early Alzheimer's disease and mild cognitive impairment, as expected. There was considerable overlap of the extent of binding between patients with mild cognitive impairment and controls. Some investigators have even suggested (albeit without definitive evidence) that 2- ^{18}F FA might have a predictive potential in patients with mild cognitive impairment at risk for developing Alzheimer's disease. The evidence for this is not yet available, however.

Studies investigating 2- ^{18}F FA continue, with attempts being made to improve the measurements of metabolites. A recent study¹⁰ showed that solid-phase extraction methods are less time consuming than high-performance liquid chromatography and might be more suitable for 2- ^{18}F FA measurements during long PET investigations.

THE NEED FOR NEW NACHR RADIOLIGANDS FOR THALAMIC AND EXTRATHALAMIC BINDING

There are two main considerations for the need for new tracers beyond 2- ^{18}F FA and 6- ^{18}F FA. First, the widest used nicotinic cholinergic radiotracer at present, 2- ^{18}F FA, which has a reasonable binding potential value of approximately 2 in the thalamus, displays the highest binding region for $\alpha_4\beta_2$ -nAChRs, but below 1 for cortical and subcortical regions. This quantification would be further compromised in the absence of up-regulation of the nicotinic cholinergic system by smoking or a reduction in binding in a neurodegenerative disorder, such as Alzheimer's disease.

A further problem is the kinetics of the radiotracer in cortical, subcortical (striatal), and thalamic brain areas. For example, with 2- ^{18}F FA the radiotracer reaches steady state either with a bolus or definitively with constant-infusion methods. However, in the thalamic areas, which are of considerable interest in some disorders, including smoking, there is a need for long infusions. Sometimes it takes up to 6 or 8 hours to reach the steady state, and bolus-injection studies—while performed for as little as 2 to 3 hours—are not ideal and undoubtedly include compromises in the mathematical modeling of the thalamic regions.

New radiotracers to study the thalamus are needed that are more reversible during reasonable PET scan times, such as 90 minutes to 2 hours. Similarly, in the cortical region, higher binding potentials are needed even if they reach a steady state during this time. Examples of some approaches include radiotracers such as ^{18}F NIDA522131, which was designed to image extra thalamic regions.^{31,32} In comparisons, this radiotracer had higher in vitro affinity and increased uptake with greater distribution volumes than 2- ^{18}F FA, especially in extra thalamic areas. The increase in brain binding of ^{18}F NIDA522131 was about four times greater than 2- ^{18}F FA in multiple brain regions, including the cingulate cortex, frontal cortex, thalamus, mid-brain and, to a less extent, putamen. However, ^{18}F NIDA522131 exhibits evidence for even less-reversible binding than that of 2- ^{18}F FA and requires more than 6 hours of scanning.

It has been hypothesized²⁰ that some of the slow entry into the brain of radiotracers, such as 2- ^{18}F FA or 6- ^{18}F FA, may be related to lipophilicity, affinity, and possibly a slow-off rate. It was documented that although peak activity may occur between 80 and 100 minutes in the brain with 2- ^{18}F FA, there was still a slow approach to steady state (tissue plasma ratios). Five hours after injection, a steady state still was not reached in the thalamus. Similarly, 6- ^{18}F FA

required at least 4 hours of scanning time and 5- $[^{123}\text{I}]$ IA at least 4 hours. All suffer from failure to achieve a steady state in various brain regions of interest.

It has been proposed²⁰ that two classes of radiotracers for nAChR are needed. These would include one group with relatively rapid brain kinetics to allow facile quantitation in the brain regions with the highest binding, such as the thalamus. A second group would require high binding in extra thalamic regions, presumably by using higher affinity radioligands. This latter group would be unlikely to reach a steady state in higher binding regions, but would be more useful in regions such as the cortex. The radiopharmaceuticals in the second group would be used for studies of neurodegenerative disorders, such as Alzheimer's disease, where cortical changes are most important. The radiopharmaceuticals in the first group would have higher binding in the thalamus and might play an important role in nicotine addiction.

PET NACHR RADIOLIGANDS WITH OPTIMIZED BRAIN KINETICS

Development of nAChR PET radioligands has a long history and it has been reviewed in several publications.^{20–25,33,34} In summary, at the moment $[^{11}\text{C}]$ nicotine, 2- $[^{18}\text{F}]$ FA, and 6- $[^{18}\text{F}]$ FA are the only available radioligands for imaging of nAChRs in human subjects. $[^{11}\text{C}]$ Nicotine is not a very suitable radioligand for PET quantification of nAChRs because of its low specific and high nonspecific binding and its very rapid metabolism. The slow brain kinetics of 2- $[^{18}\text{F}]$ FA and 6- $[^{18}\text{F}]$ FA lead to lengthy and expensive imaging studies that cannot be tolerated by patients and afforded by most PET centers. In addition, 2- $[^{18}\text{F}]$ FA and 6- $[^{18}\text{F}]$ FA exhibit relatively low binding potential values in animal and human brains.

The drawbacks of existing nAChR radioligands hamper the PET imaging research of $\alpha_4\beta_2$ -nAChR, the major class of cerebral receptors that is directly linked to various central nervous system (CNS) disorders. On the other hand, these drawbacks are the driving force of development of better PET radioligands for imaging nAChRs. The aim of these recent studies is to synthesize radioligands with an adequately high binding-potential value ($\text{BP}^{\text{Thalamus}} \geq 2$) that is normally considered a minimum requirement for quantitative PET studies. Most importantly, the expected radioligands have to rapidly reach a steady state in various brain regions and require a scanning time of less than or equal to 2 hours, which is regarded as the most practical time for human application.

The development of nAChR radioligands that are superior to 2- $[^{18}\text{F}]$ FA was based on the idea that the binding affinity (K_i) and lipophilicity ($\log D$) values are the two major factors that affect the brain kinetics and binding potentials of in vivo CNS nAChR radioligands. The most interesting results in the last few years involved the radiolabeled analogs of (i) A-84,543/A-85,380, high-affinity nAChR ligands that have been synthesized by Abbott Laboratories³⁵ and (ii) epibatidine, a very high affinity nAChR agonist that was discovered by National Institutes of Health scientists in Ecuadorian frogs (Fig. 3).³⁶

Radiolabeled Analogs of A-84,543 and A-85,380

Structurally similar pyridyl ethers A-84,543 and A-85,380 were attractive leads for discovery of new nAChR radioligands because these leads exhibit high binding affinity and excellent $\alpha_4\beta_2$ -nAChR subtype selectivity. 2- $[^{18}\text{F}]$ FA and 6- $[^{18}\text{F}]$ FA (see Fig. 1), the only available nAChR radioligands that are approved for human studies, are derivatives of A-84,543 and A-85,380, suggesting that new analogs of these leads will be sufficiently safe for human application.

$[^{11}\text{C}]$ Me-PVC

(*S,E*)-2-chloro-5-((1- $[^{11}\text{C}]$ methylpyrrolidin-2-yl)methoxy)-3-(2-(pyridin-4-yl)vinyl)pyridine ($[^{11}\text{C}]$ Me-PVC) (see Fig. 2)^{37–39} was one of the first attempts to resolve the issue of the slow

brain kinetics of 2-[¹⁸F]FA, which were thought to be because of its high polarity. [¹¹C]Me-PVC exhibits substantially greater lipophilicity than 2-[¹⁸F]FA and was expected to display better blood-brain barrier permeability. Because of the high binding affinity of [¹¹C]Me-PVC, this radioligand specifically labels nAChRs in vivo with a reasonable binding-potential value. In the Rhesus monkey, the brain kinetics of [¹¹C]Me-PVC are optimally reversible (see Fig. 2). After a bolus injection of [¹¹C]Me-PVC, only 90 to 120 minutes of scanning is necessary to obtain quantifiable PET data in a Rhesus monkey.³⁹ Despite the promising results in animals, [¹¹C]Me-PVC has never been used in human subjects because its binding potential value is slightly lower than that of 2-[¹⁸F]FA and the authors have chosen to hunt for a radioligand with equally optimal brain kinetics but greater binding potential.

[¹¹C]JHU85208, [¹¹C]JHU85157 and [¹¹C]JHU85270

As an attempt to overcome the problem of the low binding-potential value of [¹¹C]Me-PVC, three analogs of this compound ([¹¹C]JHU85208, [¹¹C]JHU85157, and [¹¹C]JHU85270) (see Fig. 2) have been synthesized.^{40,41} All three have better binding affinity and slightly lower lipophilicity than [¹¹C]Me-PVC. All three radioligands demonstrated excellent imaging properties in rodents with high accumulation of radioactivity in the nAChR-rich thalamus, moderate uptake in the cortex, a region with a medium density of nAChRs, and lowest uptake in the nAChR-poor cerebellum (Fig. 4).

Inspired by the successful rodent imaging results, the Johns Hopkins group studied these three radioligands, [¹¹C]JHU85208, [¹¹C]JHU85157, and [¹¹C]JHU85270 in baboons.⁴⁰ The kinetics of the radioligands in the baboon brain were reversible (see Fig. 2) and only 2 hours were required for the radioligands to reach a steady state in the baboon thalamus. Unexpectedly, the thalamic binding potentials of [¹¹C]JHU85208, [¹¹C]JHU85157, and [¹¹C]JHU85270 were lower than that of 2-[¹⁸F]FA (0.7–0.8 versus 2). Metabolite analysis of [¹¹C]JHU85208, [¹¹C]JHU85157, and [¹¹C]JHU85270 demonstrated that, like their analog [¹¹C](–)nicotine that metabolizes to lipophilic [¹¹C]cotinine, in baboon plasma all three ¹¹C-radioligands generated oxidative lipophilic metabolites that are likely to penetrate the blood-brain barrier and increase nonspecific binding. Because the binding-potential values are relatively low there are no current plans to pursue [¹¹C]JHU85208, [¹¹C]JHU85157, and [¹¹C]JHU85270 as radioligands for human imaging.

[¹⁸F]Nifene

The development of 3-((2,5-dihydro-1H-pyrrol-2-yl)methoxy)-2-[¹⁸F]fluoropyridine ([¹⁸F]nifene)^{42,43} was justified by the hypothesis that an analog of 2-[¹⁸F]FA with slightly reduced binding affinity might display more rapid brain kinetics. The binding affinity of [¹⁸F]nifene is lower than that of 2-[¹⁸F]FA (see Fig. 2). As was expected, the brain kinetics of [¹⁸F]nifene in the Rhesus monkey brain is highly reversible (see Fig. 2). Unfortunately, the moderate thalamus/cerebellum ratio value of 2.2 of [¹⁸F]nifene in monkey is the price paid for the rapid brain kinetics of this radioligand with relatively low binding affinity. The authors are not aware of any further in vivo studies with [¹⁸F]nifene in animals or human subjects.

[¹⁸F]- and [¹¹CH₃]-(*S*)-3-(6-fluorohex-1-ynyl)-5-((1-methylpyrrolidin-2-yl)methoxy) pyridines

The title isotopomer-radioligands (see Fig. 2) was a part of a medicinal chemistry project directed toward the synthesis of ligands with high β_2/β_4 -nAChR subtype selectivity ($K_i^{\alpha_4\beta_2} = 0.95$ nM, $K_i^{\alpha_3\beta_4} = 88,000$ nM).⁴⁴ In baboon PET imaging studies, the radioligands⁴⁵ specifically labeled nAChRs in the nAChR-rich thalamus with optimally reversible brain kinetics (see Fig. 2). The thalamus/cerebellum ratio reached a value of 2.5 at 1 hour after injection. It is unlikely that [¹⁸F]- or [¹¹CH₃]-(*S*)-3-(6-fluorohex-1-ynyl)-5-((1-methylpyrrolidin-2-yl)methoxy)pyridines will be useful for $\alpha_4\beta_2$ -subtype nAChR imaging because both radioligands manifested high uptake of radioactivity in the nAChR-poor striatum.

After injection of the radioligands into baboons, the uptake of the radioactivity in the striatum was not diminished by injection of nicotine. This observation suggests that striatum binding is not mediated by nAChRs and can be explained by the appearance of radiolabeled metabolites that are ligands of another CNS receptor.

Radiolabeled Analogs of Epibatidine

Epibatidine is a very high affinity agonist of nAChRs that exhibits little nAChR-subtype selectivity and is notorious for its acute toxicity. In the past, many PET-labeled epibatidine analogs have been developed (see for review²⁰). Several of these compounds exhibited excellent imaging properties but they were too toxic for human application. The recent success in the development of radiolabeled analogs of epibatidine for PET imaging of nAChRs is largely indebted to the discovery of derivatives of epibatidine analogs with properties of antagonists of nAChR.^{46,47} This discovery initiated a series of research studies by several groups that are described below.

(±)- [¹¹C]NMI-EPB

The synthesis of 7-methyl-2-exo-(3'-iodo-5'-pyridinyl)-7-azabicyclo[2.2.1]heptane ((±)-NMI-EPB) was first published by Carroll and colleagues.⁴⁷ Unlike many other epibatidine analogs that are highly toxic nAChR agonists, (±)-NMI-EPB with a bulky substituent in position 5 of the pyridine ring is a nAChR antagonist with high binding affinity⁴⁷ (Fig. 5). Being a nAChR antagonist, (±)-NMI-EPB should not be as toxic as epibatidine. It was expected that (±)-NMI-EPB would be sufficiently safe for in vivo experiments. Thus, in the functional in vivo studies⁴⁷ up to 0.8 mg/kg (1,250 nmol/kg – 2500 nmol/kg) of (±)-NMI-EPB was injected into mice. In further experiments the same group radiolabeled (±)-NMI-EPB with ¹¹C and [¹¹C] (±)-NMI-EPB has been studied by PET in baboon.⁴⁸ The radioligand specifically labeled nAChRs in the baboon brain with a very good ratio of specific/nonspecific binding (see Fig. 5). Because the time-uptake curves in the baboon brain regions exhibit a 5-minute peak, it was reasonable to hypothesize that [¹¹C](±)-NMI-EPB might manifest rapid brain equilibrium and that the radioligand would potentially be more advantageous than 2-[¹⁸F]FA and 6-[¹⁸F]FA.

The binding affinity of enantiomers of N-methylepibatidine manifests little enantioselectivity,⁴⁹ but the analogs of N-methylepibatidine with large substituents in the pyridine ring are highly enantioselective.⁵⁰ Therefore, one could anticipate that (–)- and (+)-enantiomers of (±)-NMI-EPB should be enantioselective and a more potent enantiomer of ¹¹C-NMI-EPB might exhibit better PET imaging properties than those of [¹¹C](±)-NMI-EPB. Preparation of (+)-NMI-EPB and (–)-NMI-EPB was reported and the binding affinity of (–)-NMI-EPB was 30 times greater than that of (+)-NMI-EPB.⁵¹ A rodent study with [¹¹C](–)-NMI-EPB has confirmed that the radioligand labels cerebral nAChRs with a high level of specificity.⁵¹ Unfortunately, the baseline baboon PET imaging showed that [¹¹C](–)-NMI-EPB does not reach a steady state in the thalamus within 90 minutes after administration and its kinetics are too slow for a ¹¹C-radioligand with a 20-minute half-life. The previously described rapid brain kinetics of [¹¹C](±)-NMI-EPB are likely to correspond to the superimposition of the slow kinetics of [¹¹C](–)-NMI-EPB and very fast nonspecific uptake of less active [¹¹C](+)-NMI-EPB. Therefore, the authors of the article suggested that neither [¹¹C](–)-NMI-EPB nor [¹¹C](±)-NMI-EPB are suitable for quantitative human PET imaging.⁵¹

[¹⁸F]FPhEP and [¹⁸F]F2PhEP

Two more $\alpha_4\beta_2$ -nAChR selective⁵² epibatidine analogs from Carroll and colleagues,⁵³ 2-(6-fluoro-5-phenylpyridin-3-yl)-7-aza-bicyclo[2.2.1]heptane, FPhEP (K_i = 240 pM) and 2-(6-fluoro-5-(4-fluoro-phenyl)pyridin-3-yl)-7-aza-bicyclo[2.2.1]heptane, F2PhEP (K_i = 29 pM) (see Fig. 5) were of interest for PET.^{52,54,55} The researchers from Orsay radiolabeled both compounds with ¹⁸F and studied [¹⁸F]FPhEP and [¹⁸F]F2PhEP in baboons.^{54,55} Both

radioligands readily penetrated the blood-brain barrier, showing more rapid brain kinetics than those of 2-[¹⁸F]FA. As evidence of the specific in vivo binding of [¹⁸F]FPhEP in the baboon brain, its uptake was blocked by injection of nicotinic ligands. Unfortunately, the binding-potential value of [¹⁸F]FPhEP (see Fig. 5) was too low for further investment in this radioligand.

The binding affinity of F2PhEP is one order of magnitude greater than that of FPhEP, but [¹⁸F]F2PhEP has thalamic uptake that is lower than of [¹⁸F]FPhEP.^{54,55} In contrast, the shape of the time-activity curves in different brain regions reflected the affinity of the ligands. [¹⁸F]FPhEP displayed similar time-activity curves in all regions of interest (except white matter), whether or not the region is receptor rich or poor, whereas for [¹⁸F]F2PhEP uptake was substantially greater in the thalamus than in the cerebellum. Surprisingly, the uptake of [¹⁸F]F2PhEP in the cortex, the region with substantial density of nAChRs, was lower than uptake in the nAChR-poor cerebellum. The radioligand [¹⁸F]F2PhEP also presented another surprise in the blocking studies when it did not show a significant reduction of radioactivity accumulation after injection of nicotine. The authors of the article⁵⁴ concluded that the radiotracers did not fulfill the widely adopted criteria for a quality PET radioligand. It is likely that both radioligands are too lipophilic (see Fig. 5) and their nonspecific binding is too high for a successful radioligand.

[¹⁸F](–)NCFHEB

Enantiomers of the radiofluorinated homolog of epibatidine, 6-(6-[¹⁸F]fluoropyridin-3-yl)-8-aza-bicyclo[3.2.1]octane, (see Fig. 5), (–)- [¹⁸F]NCFHEB, and (+)-NCFHEB were reported in several recent publications.^{56–58} Both compounds display high $\alpha_4\beta_2$ -nAChR and low $\alpha_3\beta_4$ -nAChR in vitro binding affinity and excellent specific radiolabeling of nAChRs in mice.⁵⁶ The same group performed a substantial number of PET studies and compared the porcine brain kinetics of (–)-[¹⁸F]NCFHEB and (+)-NCFHEB with 2-[¹⁸F]FA to evaluate the potential of the new radioligands for human neuroimaging. Several animals received an additional intravenous injection of the nAChR agonist A81418 to confirm the specific binding of the radioligands. Both enantiomers of [¹⁸F]NCFHEB showed a higher brain uptake and better binding-potential values than 2-[¹⁸F]FA (see Fig. 5). In the piglet thalamus (+)-[¹⁸F]NCFHEB, (–)-[¹⁸F]NCFHEB and 2-[¹⁸F]FA manifest a peak uptake at 142 minutes, 80 minutes, and 68 minutes, correspondingly, suggesting relatively slow brain kinetics of the new radioligands. Interestingly, the thalamus/olfactory bulb ratio had reached its peak for (–)-[¹⁸F]NCFHEB earlier than for 2-[¹⁸F]FA. This observation led the investigators to the opinion that the equilibrium of specific binding of (–)-[¹⁸F]NCFHEB was reached earlier than that of 2-[¹⁸F]FA and, therefore, PET imaging properties of (–)-[¹⁸F]NCFHEB may be potentially superior to 2-[¹⁸F]FA. The authors look forward to further development of (–)-[¹⁸F]NCFHEB for PET imaging of nAChRs in nonhuman primates and, perhaps, in human subjects.

Development of [¹⁸F]AZAN ([¹⁸F]JHU87522)

High demand for a better radioligand for imaging $\alpha_4\beta_2$ -nAChRs inspired the authors group to exert a major synthetic effort to develop a nicotinic-receptor ligand with optimized imaging properties. As the result of these studies, an ¹⁸F-labeled nAChR antagonist (–)-2-(6-[¹⁸F]fluoro-2,3'-bipyridin-5'-yl)-7-methyl-7-aza-bicyclo[2.2.1]heptane ([¹⁸F]JHU87522 or [¹⁸F]AZAN) was obtained that showed evidence of better PET imaging properties than 2-[¹⁸F]FA. These properties include brain kinetics that require only 1.5 hours of data acquisition after bolus administration, good binding-potential values (2.5–4), very high total brain uptake, absence of lipophilic metabolites, a good safety profile, and simple radiosynthesis, with more details shown below.

The preliminary studies⁵⁰ suggested that ¹⁸F-radiolabeled derivatives of dipyrindyl analogs of epibatidine show excellent potential for PET. The structure-activity relationship studies demonstrated that the high binding-affinity value of dipyrindyl analogs of epibatidine can be optimized within the subnanomolar range by modification of substituents in the external pyridine ring, and by the change of the connecting position of the ring. Because of this, the authors had a large degree of freedom in optimization of binding affinity and lipophilicity. The authors were convinced that the best preliminary radioligand, [¹⁸F](–)-JHU86358, which displayed very high binding affinity and binding potential but relatively slow brain kinetics, could be optimized toward a compound with faster brain kinetics and a sufficient binding potential value if the binding affinity was slightly reduced. Three additional isomers of [¹⁸F](–)-JHU86358, namely [¹⁸F](–)-JHU86430, [¹⁸F](–)-JHU86428, and [¹⁸F](–)-JHU87522 with similar physical-chemical properties but reduced binding affinities (see Fig. 5) have been synthesized. [¹⁸F](–)-JHU86430 exhibited almost the same K_i value as that of [¹⁸F](–)-JHU86358 and both radioligands showed comparable PET imaging properties.

The next isomer, [¹⁸F](–)-JHU86428 ((–)-2-(2'-[¹⁸F]fluoro-3,3'-bipyridin-5-yl)-7-methyl-7-aza-bicyclo[2.2.1]heptane, [¹⁸F]XTRA) with slightly reduced binding affinity (see Fig. 5), peaked in the baboon thalamus at 75 minutes after injection. This compound had excellent binding-potential values and required about 2.5 hours scanning in thalamus and 1.5 hours scanning in the cortex after bolus injection.⁵⁹ [¹⁸F]XTRA holds promise for imaging extrathalamic nAChRs in human beings.

The isomer [¹⁸F](–)-JHU87522⁵⁹ ((–)-2-(6-[¹⁸F]fluoro-2,3'-bipyridin-5'-yl)-7-methyl-7-aza-bicyclo[2.2.1]heptane, [¹⁸F]AZAN) had the lowest binding affinity within the series (see Fig. 5) and gave the most interesting results in baseline and blockade baboon PET studies. The radioligand requires only 90 minutes of scanning to reach a steady state. In addition to the rapid brain kinetics, [¹⁸F]AZAN displays a higher binding-potential value than 2-FA. [¹⁸F]AZAN also exhibits a 200% greater total brain uptake than 2-[¹⁸F]FA and an absence of lipophilic metabolites. The radiosynthesis of [¹⁸F]AZAN is a one-step radiolabeling that is simpler than the two-step radiosynthesis of 2-[¹⁸F]FA.

The inhibition binding assay studies with various nAChR subtypes demonstrated that AZAN is a highly selective $\alpha_4\beta_2$ -nAChR ligand, whereas a functional assay in vitro showed that AZAN exhibits properties of a potent antagonist of the $\alpha_4\beta_2$ -nAChR subtype. Preliminary behavioral toxicologic studies in mice demonstrated that AZAN exhibits acute toxicity comparable to that of 2-FA.⁵⁹ Radiation dosimetry and toxicology studies with AZAN will establish whether or not [¹⁸F]AZAN is safe for PET imaging in human beings.

SUMMARY

PET imaging of $\alpha_4\beta_2$ -nAChRs in human subjects is a vibrant field of contemporary neuroscience and nuclear medicine. Nicotinic imaging studies in smoking, epilepsy, attention deficit hyperactivity disorder, depression, schizophrenia, cognition, behavior, memory, and in research of aging, cognitive impairments, and dementia attract the attention of many researchers. The nAChR radioligands that are currently available for PET manifest slow brain kinetics and low binding potentials that prevent widespread use of PET imaging research of nAChR in human beings.

To address the problems with existing PET radioligands for imaging of nAChRs, especially the most common one 2-[¹⁸F]FA, several research groups have worked to develop novel compounds with improved brain kinetics. One new compound is (–)-[¹⁸F]NCFHEB, which demonstrated PET imaging properties that are superior to 2-[¹⁸F]FA in pigs. Further

experiments with this radioligand that include PET imaging in nonhuman primates and safety studies are needed to qualify (–)-[¹⁸F]NCFHEB as a potential candidate for human imaging.

The most recent candidate, radioligand [¹⁸F](–)-JHU87522 ([¹⁸F]AZAN), exhibited all the necessary properties that are required for a successful PET radiotracer for imaging of nAChRs. ⁵⁹ [¹⁸F]AZAN specifically labels cerebral nAChRs with high binding potential and its brain uptake is substantially greater than that of 2-[¹⁸F]FA. Most importantly, [¹⁸F]AZAN requires only 90 to 100 minutes of PET scanning to obtain quantifiable receptor data. In addition, [¹⁸F]AZAN is a β₂-subtype selective nAChR antagonist with low acute side effects in animals. The radiosynthesis of [¹⁸F]AZAN is simpler than that of 2-[¹⁸F]FA. All of these properties suggest that [¹⁸F]AZAN is a substantially better radioligand than 2-[¹⁸F]FA, the most popular radioligand for PET imaging of nAChRs in the human brain. The safety studies currently being performed with [¹⁸F]AZAN will determine whether [¹⁸F]AZAN can replace 2-[¹⁸F]FA in the PET clinic.

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Fig. 1.

The currently available nAChR PET radioligands for human studies.

Radioligand	Name	logD _{7.4}	K _i , nM	BP	Peak time in thalamus (required scanning time), min
2-[¹⁸ F]FA	-1.4	0.1 - 1.3	2	120 (480)
[¹¹ C]Me-PVC	2	0.06	1.7	20 (90)
[¹¹ C]JHU85208	1.8	0.06	0.7	10 (120)
[¹¹ C]JHU85157	2.2	0.05	0.8	10 (120)
[¹¹ C]JHU85270	1.6	0.06	0.7	30 (120)
[¹⁸ F]Nifene	-0.5	0.5	1.1	5 (90)
(S)-3-(6-[¹⁸ F]fluorohex-1-ynyl)-5-((1-methylpyrrolidin-2-yl)methoxy)pyridine	1.4	1	1 ^a	4 (60)

Fig. 2.

2-[¹⁸F]FA and its new analogs with improved imaging properties. ^aHigh accumulation of radioactivity in striatum suggests the presence of active radiolabeled metabolites.

Fig. 3.

Lead compounds for the recent development of nAChR radioligands.

Fig. 4.
Small animal PET image (summed 20–90 minutes) of [^{11}C]JHU85157 in the rat brain.
Courtesy of C.J. Endres, PhD, Baltimore, MD.

Radiolabeled analog of epibatidine	Code name	logD _{7.4} ^c	K _i , nM ^d	BP	Peak time in thalamus (required scanning time), min
(±)-NMI-EPB	1.1	0.03	1.5-2.5 Th	5 (>90-120) ^a
[¹⁸ F]FPhEP	2.9	0.24	~0.5 Th	20 (~90-120) ^a
[¹⁸ F]F2PhEP	3.0	0.03	~2 Th	45 (~180) ^a
[¹⁸ F](+)-NCFHEB	-1.4	0.06	7.6 Th	142 (~300) ^b
	[¹⁸ F](-)-NCFHEB		0.1	2.5 Th	80 (~100) ^b
[¹⁸ F](-)-JHU86358	0.5	0.05	>7 Th 1.1 ^{Cx}	>180 (>180) ^a
[¹⁸ F](-)-JHU86430	0.7	0.05	>7 Th 1.0 ^{Cx}	160 (>180) ^a
[¹⁸ F](-)-JHU86428 ([¹⁸ F]XTRA)	0.7	0.1	7.3 Th 1.3 ^{Cx}	65 (120-150) ^a
[¹⁸ F](-)-JHU87522 ([¹⁸ F]AZAN)	1.0	0.3	2.1-4.8 Th 0.6-1.0 ^{Cx}	10-25 (90) ^a

Fig. 5. PET imaging properties of new radiolabeled analogs of epibatidine. ^aBaboon; ^bPiglets; ^cLipophilicity value (logarithm of partition coefficient between n-octanol and water at pH 7.4); ^dInhibition binding affinity. Cx = cortex, Th = thalamus.

Table 1

PET Imaging of nAChR in human subjects

PET/SPECT Radiotracer	Number of Patients	Single Scan or Dynamic	Arterial Line	Bolus or Continuous Infusion	Reference
(-)- [¹¹ C] nicotine	5 subjects	Dynamic; baseline and challenge	Yes	Bolus	3
2- [¹⁸ F]FA	6 healthy controls	Single whole-body scan and dynamic brain scan	No	Bolus	5
2- [¹⁸ F]FA	3 healthy controls	Single whole-body scan	No	Bolus	6
2- [¹⁸ F]FA	7 healthy controls	Dynamic	Yes	Bolus	7,8
2- [¹⁸ F]FA	8; 4 of which were smokers	Dynamic	Yes	Bolus plus infusion	9
2- [¹⁸ F]FA	97 (12 healthy controls)	Single	Yes	Bolus	10
2- [¹⁸ F]FA	15 AD patients; 14 age-matched controls	Single scan	No	Slow intravenous bolus injection	11,12
2-FA	10 normal volunteers	Dynamic	Yes	Bolus	13
2- [¹⁸ F]FA; [¹⁸ F]FDG	8 nonsmoking ADNFLE patients; 7 age-matched controls; 5 patients underwent additional FDG PET experiment	Dynamic	No	Bolus	14
2- [¹⁸ F]FA	17 AD patients; 6 w/amnesic MCI; 10 normal aged healthy controls	Dynamic	Yes	Bolus	15
2- [¹⁸ F]FA	7 smokers; 7 nonsmokers	Dynamic	Yes	Continuous infusion	16
6- [¹⁸ F]FA	5 nonsmokers	Dynamic (2-hour scan) 4-hour study	Yes	Bolus	17
5- [¹²³ I]IA	6 nonsmokers, 6 light smokers	Dynamic	Yes	Bolus and continuous infusion	18

Abbreviations: AD, Alzheimer's disease; ADNFLE, autosomal dominant nocturnal frontal lobe epilepsy; FDG, [¹⁸F]-2-fluoro-deoxy-D-glucose; MCI, mild cognitive impairment; SPECT, single-photon emission computed tomography.