Fundamental studies on radiotracers intended for receptor imaging in dementia

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CHAPTER 1

INTRODUCTION, AIMS AND OUTLINE OF THE THESIS

Introduction
According to the ICD-10, dementia is a syndrome due to disease of the brain, usually of a chronic or progressive nature, in which there is disturbance of multiple higher cortical functions, whereas no disturbances in consciousness are present. The impairments of cognitive function are commonly accompanied by deterioration in emotional control, social behavior, or motivation.

The prevalence of dementia is highly age-dependent. Data from the RIVM (2003) indicate that the prevalence of dementia in The Netherlands is less than 1% of individuals aged between 60 and 70 years, but 32% for males and 36% for females aged 85 years or more. Due to ageing of the population, the prevalence of the syndrome will rise substantially in the near future and will become a major healthcare problem.

Although no cures for most types of dementia are available and although pharmaceutical therapies only show modest effects up till now, a correct diagnosis favors all aspects of care for these patients. Today, the role of medical imaging in the diagnosis of dementia is limited, but will become much more important once improved therapies have been developed. In-vivo imaging procedures, for instance molecular imaging techniques, are likely to assist increasingly in the differentiation between the various types of dementia, the evaluation or prediction of treatment response, or in the development of new pharmaceutical therapies for dementia.

The aims of the present thesis were to explore novel candidate radiotracers intended for imaging of dementia by means of molecular imaging techniques, and to evaluate the value of existing radiotracers that are currently being used, or could potentially be used for imaging of dementia.

Types of dementia
Many causes or diseases may underlie the syndrome of dementia, but the vast majority of dementia cases result from four categories of diseases. Alzheimer’s dementia or Alzheimer’s disease (AD) is the most common
type of dementia, accounting for approximately 60% of all dementia cases. Lewy body disorders such as dementia with Lewy bodies (DLB) and Parkinson’s disease related dementia (PDD) together account for 15% of the dementia cases. Vascular dementia is estimated to be the cause of dementia in approximately 20% of the cases and frontotemporal dementia in 5%, but the latter two will not be discussed in this thesis.

**Dementia of the Alzheimer type**
The most common type of dementia was first described by the German psychiatrist Alois Alzheimer in 1906, who performed autopsy on the brain of one of his former patients, Auguste Deter, who had suffered from an early type of dementia in the years before her premature death at the age of 56. Alzheimer was the first to describe the neuropathological hallmarks of the disease by microscopic examination of the brain. Although initially described as ‘presenile dementia’, the type of dementia that was referred to by Alois Alzheimer is presently known as dementia of the Alzheimer type or AD. This disease is characterized clinically by a deterioration of memory and cognitive disturbances such as language and visuospatial deficits, which usually is insidious in onset. The condition is progressive and results in behavioral and functional impairments that interfere increasingly with daily tasks. Other symptoms may include motor and sensory abnormalities, gait disturbances and seizures, although these symptoms are usually seen in advanced stages of the disease. The diagnosis of the disease is based on clinical symptoms and patient history, but a definitive diagnosis can only be made by histology of brain sections on autopsy. Clinical criteria are provided by the Diagnostic and Statistical Manual of Mental Disorders, or the criteria of the National Institute of Neurologic and Communicative Disorders and Stroke-Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA). The sensitivity and specificity of such criteria are typically less than 80% for the diagnosis of probable AD.

**Histopathologic features of AD**
Histopathologically, the diagnosis of AD is obtained from the presence of cortical amyloid containing neuritic plaques (or senile plaques), cerebrovascular plaques and neurofibrillary tangles (NFTs), originally described by Alois Alzheimer, in at least neocortical areas and medial temporal lobe structures. The likelihood of AD can be assessed histopathologically.
by either the CERAD (Consortium to Establish a Registry for Alzheimer’s Disease) score or the Braak and Braak stage.

The amyloid cascade hypothesis of AD

Amyloid-β (Aβ), a cleavage product of a larger transmembrane protein named amyloid precursor protein (APP) which is coded on chromosome 21, is the principal component of soluble Aβ-oligomers, cerebrovascular and neuritic plaques. Cleavage of APP is performed by protease enzymes called secretases (α, β, and γ-secretase), which normally is a non-amyloidogenic event if α- and γ-secretase are involved in the cleavage process, and is referred to as the secretory pathway. Potentially amyloidogenic is the non-secretory pathway where isoforms of Aβ with a 40–42 amino acid sequence in length are formed by cleavage of the precursor protein by initially the β-secretase and subsequently the γ-secretase of which the latter depends on the presenelin proteins PS1 and PS2 to perform its actions. Normally, degradation and clearance of Aβ in the brain prevent aggregation of the peptide. Mutations in APP, PS1 and PS2 are associated with rare hereditary early types of AD, and provide evidence for the importance of these proteins in the formation of soluble Aβ-oligomers or plaques. Less rare, and supposedly the most important contributor to genetic predisposition for sporadic AD is the presence of the apolipoprotein 4 ε4 allele, coding for ApoE4 and thought to be a dysfunctional isoform of the cholesterol transporter ApoE, which is associated with fibrillisation of Aβ into soluble Aβ-oligomers and the formation of plaques.

The most common isoforms of Aβ peptide are the 40 and 42 amino acid isoforms Aβ40 and Aβ42. Of these two, Aβ42 has a tendency to aggregate into soluble oligomers and insoluble amyloid fibrils that give rise to neuritic plaques, which have long been known to correlate with severity of dementia. Although the Aβ40 isoform has been associated with cerebrovascular plaques, beneficial effects of this isoform have been described in that it antagonizes Aβ42 aggregation and thus, the ratio between concentrations of Aβ42 and Aβ40 seems to be of importance in plaque formation. Aβ42 may trigger misfolding of other Aβ isoforms which contributes to plaque formation. The amyloid cascade hypothesis for AD initially considered the neuritic plaques to be responsible for disruption of neuronal circuitry due to neurotoxicity, increased oxidative stress and induction of inflammatory responses, a process that eventually leads to dementia in the patient. However, more recent evidence suggests that accumulation of soluble Aβ-oligomers might be of equal importance in this respect.
Neurofibrillary tangles

Another observation by Alois Alzheimer in the brain of his patient was the existence of NFTs. These intra-neuronal tangles are formed by abnormally processed tau protein\textsuperscript{24}, and are found also in other neurodegenerative diseases\textsuperscript{25}. Whether these tangles are a cause or a result of AD is still under debate\textsuperscript{9}. Being an axonal protein, tau normally functions as a stabilizing protein for microtubules but hyperphosphorylation of the protein causes sequestration of normal tau and other microtubule associated microtubule-proteins\textsuperscript{9,24}. Hyperphosphorylation is balanced by dephosphorylation which converts the tau protein back to its normal state. However, imbalance between hyper- and dephosphorylation may lead to a compensational neuronal increase of normal tau synthesis and packaging of the abnormally hyperphosphorylated tau protein into inert polymers, which in the end may result in the NFT. These cytoplasmic accumulations lead to disturbances of the axonal transport, neuronal and synaptic dysfunction and eventually of degeneration of neurons\textsuperscript{24} which contributes to the pathogenesis of dementia.

Neurotransmitter systems in AD

Due to the neurodegenerative events in AD, practically all neurotransmitter systems in the central nervous system show disturbances, although the extent of involvement varies between the several neurotransmitter systems. Of particular importance for this thesis is the muscarinic system, which will be discussed in detail. The nicotinic system is being discussed briefly, whereas the glutamatergic system will also be discussed briefly.

The cholinergic neurotransmitter system

The central cholinergic neurotransmitter system is one of the principal neurotransmitter systems in the central nervous system. The system is subdivided in two separate systems known as the nicotinic and muscarinic system and hence their name, nicotinic receptors have a high affinity for nicotine whereas muscarinic receptors have a higher affinity for muscarine, which is an alkaloid that was originally derived from the mushroom amanita muscaria. Both receptor systems use acetylcholine (ACh) as its neurotransmitter, which is deactivated by acetylcholinesterase in the synaptic cleft. The primary focus of this thesis is on the muscarinic system.
The muscarinic neurotransmitter system

Five distinct types of muscarinic receptors have been identified genetically (on genes m1-5) and localized in the mammalian brain (expressed as muscarinic receptor M1-5)\textsuperscript{26}. Muscarinic receptors belong to the members of G-protein coupled receptors and have seven transmembrane domains\textsuperscript{27}. The M\textsubscript{1} receptor is localized predominantly but not exclusively on the postsynaptic membrane and is the most abundantly expressed muscarinic receptor in the mammalian central nervous system. In the cholinergic system, the M\textsubscript{2} receptor is expressed predominantly but not exclusively on the membrane of presynaptic muscarinic nerve terminals\textsuperscript{28}, although its localization on the synaptic membrane seems to vary amongst brain structures\textsuperscript{29}. The M\textsubscript{2} receptor is considered to act as an autoreceptor, thereby modulating the amount of ACh in the synaptic cleft and is expressed at a density of approximately 25\% of M\textsubscript{1} receptors in most areas of the mammalian brain\textsuperscript{30,31}, although a relatively high expression is found in the hippocampus and entorhinal cortex in humans\textsuperscript{32}, and the rodent thalamus and cerebellum\textsuperscript{30}. The M\textsubscript{4} subtype, predominantly localized on the presynaptic nerve terminals\textsuperscript{29}, is expressed abundantly in cortical brain areas of rodent brain\textsuperscript{30}. The M\textsubscript{3} receptor, located predominantly postsynaptically\textsuperscript{29}, has a low rate of expression throughout the brain\textsuperscript{30}, as is the case with the M\textsubscript{5}, which has been localized with relative high densities in the substantia nigra\textsuperscript{26}.

Two major groups of cholinergic neurons are generally distinguished within the mammalian brain. The first is the magnocellular basal forebrain cholinergic system\textsuperscript{33-35} which is located in the basal forebrain within the medial septal nucleus, vertical and horizontal limb nuclei of the diagonal band of Broca, and the nucleus basalis magnocellularis (NBM; referred to as the nucleus basalis of Meynert in the human brain). These cholinergic neurons innervate the neocortical areas, the cingulate cortex, and hippocampus, amygdala, and the olfactory bulb. The second group is formed by the brainstem cholinergic neurons, originating from this brain structure in the region of the pedunculopontine tegmental nucleus and dorsolateral pontine tegmentum. This group predominantly innervates the thalamus and substantia nigra\textsuperscript{36}. Functional aspects of the basal forebrain cholinergic system include conscious awareness, attention, working memory, and a number of additional mnemonic processes\textsuperscript{35}. The brainstem cholinergic system has been shown to be involved in arousal and regulation of the sleep/wake cycle\textsuperscript{36}. 

\textit{Introduction, aims and outline}
The cholinergic hypothesis of AD

The cholinergic hypothesis was formulated approximately 25 years ago and linked cholinergic dysfunction in elderly and patients suffering from dementia to the cognitive decline that is displayed in these groups of patients. Disturbances of the cholinergic neurotransmitter system have been found consistently in the brains of patients who had suffered from AD, especially in the basal forebrain and the NBM. These disturbances have been shown to contribute to, and correlate with cognitive dysfunction. Changes of muscarinic receptor density in the cortex of AD patients have been a matter of debate ever since, but there is substantial evidence for a selective decrease of M2 receptor whereas the M1 subtype may be preserved, although the function of the latter has shown to be impaired in AD due to defective coupling to its G-protein. However, the data on muscarinic M2 receptor decreases in the brain of AD patients is mostly collected from patients with advanced disease, and it is still not entirely clear whether this also occurs in early disease.

Additionally, the cholinergic hypothesis was supported by earlier performed experiments in which memory deficits were induced by cholinergic antagonists in healthy subjects, whereas improvements in memory function could be achieved in aged and demented subjects after administration of cholinergic stimulants such as M1 agonists or acetylcholinesterase inhibitors (AChEIs). Also, in animal experiments, many cholinergic antagonists have produced deleterious effects on memory, while cholinergic agonists have shown opposite effects.

In order to compensate biochemically for the loss of ACh in the brain, cholinergic modulating medication such as AChEIs has been subjected to many clinical trials which have led to the FDA approval of rivastigmine, donepezil and galantamine for therapeutic use in AD. The AChEI tacrine was also approved, but is used rarely nowadays due to its hepatotoxic effects. On average, the effects of such therapies are small and result in an improvement of approximately 2.7 points in the midrange of the 70 point Alzheimer Disease Assessment Scale-Cognitive scale (ADAS-Cog). Importantly, these beneficial effects have only been proven for patients with mild to moderate AD, although effects on cognition in severe AD have also been described. Adverse effects of AChEIs are relatively common in patients, accounting for discontinuation of such therapies in almost one third of the cases, and include primarily nausea, vomiting and diarrhea.
**Interaction between the muscarinic system and Aβ**

Many preclinical studies have linked the cholinergic hypothesis to the amyloid hypothesis\(^68,69\). Selective stimulation of M\(_1\) receptors by agonists has been shown to shift APP cleavage from the non-secretory amyloidogenic pathway to the secretory pathway\(^70\), resulting in a decrease in Aβ\(_{42}\) in a transgenic mouse model of AD. This and other studies also showed exacerbation of Aβ and tau pathology after administration of selective M\(_1\) antagonists. Likewise, selective stimulation of the M\(_2\) receptor subtype has been shown to impair the non-secretory amyloidogenic pathway\(^71\). On the other hand, AChEIs may have favorable effects on the APP cleavage process and prevent amyloidogenic events, as was reported in several studies\(^72\).

Conversely, Aβ has shown to selectively affect the cholinergic neurotransmitter system in several ways\(^68\). Interactions have been shown on the level of ACh synthesis\(^73\), whereas passive immunization by anti-Aβ antibodies in a transgenic mouse model of AD reversed the decreased ACh release in the hippocampus of treated mice\(^74\). More direct neurotoxic effects on cholinergic neurons have also been reported\(^75\), including disturbances at the level of muscarinic receptor function or muscarinic receptor density\(^68\).

**The nicotinic neurotransmitter system in AD**

Nicotinic receptors in the brain are involved in cognitive processes such as learning and memory, and dysfunction of the nicotinic neurotransmitter system has been shown in many neuropsychiatric diseases\(^76\). Additional to the disturbances in the muscarinic neurotransmitter system, dysfunction of the nicotinic system is also known to occur in AD\(^77\), and may occur earlier in the disease than changes in muscarinic receptor density\(^78\). Of the nicotinic receptors, which act as transmitter-gated ion-channels, the α\(_4β2\) subtype is the most abundantly expressed nicotinic receptor in the brain\(^79\). Many autopsy studies have shown decreases in the density of this receptor subtype in AD\(^80,81\), and the effects of AChEIs on cognitive impairment or AD may be partially due to their actions on the nicotinic system\(^82\). In analogy to the muscarinic system, associations between nicotinic receptors and Aβ plaque formation have been identified. In this mechanism the α\(_7\) nicotinic receptor subtype, the second most abundantly expressed subtype in the brain, seems to be of particular interest\(^83\).
The glutamatergic neurotransmitter system in AD

As the principal excitatory neurotransmitter system in the central nervous system, the glutamatergic system has a key role in the processing of information, synaptic plasticity and thus learning processes and memory formation. Dysfunction of this system has been shown in AD in which an enhancement of activity accompanies and induces neuronal death. The most important receptor of the glutamatergic neurotransmitter system is the N-methyl-D-aspartate (NMDA) receptor, which acts as a gated ion channel via heterogenic assemblies of various subunits, principally the NR1 and NR2 subunits. Activation of the receptor requires binding of the neurotransmitter glutamate but also binding of a co-agonist such as glycine. Simultaneous binding of these two substances opens the ion channel, inducing an influx of preferentially Ca\(^{2+}\) ions into the neuron, until a certain membrane potential is exceeded and a voltage dependent Mg\(^{2+}\) block closes the channel.

In AD, excessive stimulation of the glutamatergic system may lead to an excessive neuronal influx of Ca\(^{2+}\) and subsequently excitotoxic neuronal death. It has been shown that A\(\beta\) is able to enhance NMDA receptor activity. Moreover, activation of the NMDA receptor by agonists has been shown to promote A\(\beta\) production and to induce a shift from the secretory to the non-secretory amyloidogenic pathway in neuronal cultures. Pharmacologic inhibition of the NMDA receptor, for instance by memantine, may therefore have neuroprotective effects. Indeed, preclinical studies showed neuroprotective effects of this substance and after successful clinical trials, demonstrating beneficial effects of memantine on the symptoms of AD patients in all stages of the disease, this drug has been approved recently for use in AD.

Other hypotheses of AD

Apart from the amyloid cascade hypothesis and the cholinergic hypothesis for AD, many other hypotheses have been proposed that could contribute to the pathogenesis of the disease. These hypotheses include disturbances in cell cycle regulating proteins, inflammatory processes, oxidative stress, mitochondrial dysfunction, aluminum toxicity and brain trauma. Although involvement of each of these mechanisms may exist, the value is still under debate.
Lewy body disorders

Lewy body disorders, the umbrella term for Parkinson’s disease (PD), PDD and DLB, are characterized primarily by degeneration of the dopaminergic, but also the central cholinergic neurotransmission systems. Parkinson’s Disease is characterized clinically principally by motor dysfunction but cognitive impairment frequently accompanies this disease. In PD, the cognitive deficits are usually restricted to disturbances in executive functioning and visuospatial tasks. In up to 60% of these PD patients, the cognitive decline evolves into a dementia, called PDD. Together, PDD and DLB account for 15% of dementia cases and both conditions are closely related, the difference being that in PDD parkinsonian features are present at least 1 year before the onset of cognitive dysfunction. The dementia that is observed in these patients is characterized by visual hallucinations, attentional impairments and fluctuations in cognitive function. PDD is usually not difficult to diagnose, as evident parkinsonian symptoms precede the development of cognitive disorders. However, a definitive diagnosis of DLB can only be established post-mortem, although recent advances in medical imaging have greatly facilitated the diagnosis of this disease in the living patient.

Differentiation between DLB and AD is of importance since, in contrast to AD patients, DLB patients are prone to develop serious adverse effects to neuroleptics (i.e. acute parkinsonian crisis). The discrimination between AD and DLB also facilitates the prediction of response to AChEIs, which have shown to be more effective in DLB patients as compared to AD patients, and leads to more accurate information to the patients and caregivers.

Imaging of dementia

The growing prevalence of dementia and its medical, social and economic impact has increasingly focused attention to these diseases in the last decades. Although to date, there are no cures available for most underlying diseases, and available therapeutic regimes only show modest effects, clinicians and caregivers are increasingly aware of the fact that an accurate diagnosis is essential for optimizing all aspects of care for these patients. Improvements of clinical diagnostic criteria have led to better ante-mortem diagnosis for various forms of dementia, but sensitivity and specificity of these criteria remain low for most forms of dementia, with the exception of the diagnosis of probable AD, which has a sensitivity and specificity of approximately 80%. Biomarkers from
blood samples would be of great value to assist with diagnosis, but are not available to date, although some cerebral spinal fluid markers show promising results.\textsuperscript{102}

**Anatomical imaging in dementia**

Imaging studies may also improve the differentiation between the dementias, but today its main clinical use is to exclude secondary and potentially treatable causes.\textsuperscript{97} Anatomical imaging such as CT or MRI of the brain may be of value for the differentiation of the diseases by imaging of regional atrophy or pathological characteristics of specific types of dementia, such as leukoariosis or vascular lesions, and may also be useful for monitoring of disease progression by morphometric techniques. Additionally, MR spectroscopy or diffusion tensor imaging may also have a role in imaging of dementia in the future.\textsuperscript{97}

**Functional imaging in dementia**

Non-invasive imaging by nuclear medicine imaging techniques such as single photon emission computed tomography (SPECT) or positron emission tomography (PET) is particularly suitable to image physiology of the brain such as blood flow or metabolism, neurochemistry and in the future, the basic molecular biology of disease.\textsuperscript{103} At present, imaging by means of SPECT or PET is used clinically to evaluate brain perfusion and metabolism by compounds such as \([^{99m}\text{Tc}]\text{HMPAO}\) or \([^{18}\text{F}]\text{FDG}\), respectively. The former imaging procedure may be useful for differentiating AD from normal controls (sensitivity 74% and specificity 91%), but the value in discriminating AD from other forms of dementia is limited (sensitivity and specificity of approximately 70-75%).\textsuperscript{104} \([^{18}\text{F}]\text{FDG}\) PET has shown to identify patients with AD with a sensitivity of 94% but with a specificity of only 73%.\textsuperscript{105} Perfusion abnormalities in frontal cerebral cortex that occur in frontotemporal dementia can be used to discriminate this dementia from AD by means of \([^{99m}\text{Tc}]\text{HMPAO}\) SPECT, with a sensitivity of 80% and a specificity of 65%.\textsuperscript{106} SPECT and PET are also used clinically to differentiate DLB from AD. \([^{123}\text{I}]\text{FP-CIT}\), a dopamine transporter tracer, can detect probable DLB with a sensitivity of 78% and a specificity of 90% by means of SPECT,\textsuperscript{107} and similar results have been reported for the PET tracer \([^{18}\text{F}]\text{DOPA}\).\textsuperscript{108} Future clinical applications of molecular imaging in dementia will not only focus on the diagnosis or discrimination of the various forms of dementia, but also on early detection in pre-symptomatic stages of the
disease. Moreover, functional imaging of specific aspects of the diseases, such as dysfunction or degenerative events in specific neurotransmitter systems, drug interaction with neuroreceptors, or neuropathological mechanisms such as Aβ formation, are to be targeted. This would allow the evaluation of experimental pharmaceutical therapies, or the prediction of therapy response.

As a result of the cholinergic hypothesis in AD and the efficacy of cholinergic drugs in cognitive disorders, considerable effort has been put in the development of radiotracers that target the cholinergic neurotransmitter system.

**Imaging of the cholinergic neurotransmitter system**

One approach to image the cholinergic system is to target its neurochemistry. In this respect, imaging of acetylcholinesterase (AChE) activity using PET tracers such as [11C]PMP, an in-vivo AChE substrate, showed decreases in patients suffering from AD as compared to controls, which correlated with the cognitive dysfunction.

Alternatively, the decrease of muscarinic receptors in post-mortem brain specimens of AD has been the inspiration for attempts to synthesize compounds that label these receptors in-vivo. The first tracers that appeared in the literature were compounds that were able to bind to muscarinic receptors non-selectively. Later, when several autopsy studies had shown differential modulation of muscarinic receptor subtypes in various neuropsychiatric diseases, including AD, development of muscarinic receptor subtype selective radiotracers was pursued.

The first tracer that was used to image muscarinic receptors in humans was RS 4-[123I]IQNB, which was based on the high affinity N-methyl analogue of quinuclidinyl benzylate (QNB), and was developed after earlier unsuccessful attempts to map the muscarinic system with radio-labeled atropine. Studies in patients suffering from dementia consistently showed a decrease of tracer binding using RS 4-[123I]IQNB SPECT, but also e.g. in patients suffering from schizophrenia. RS 4-[123I]IQNB has not demonstrated subtype selectivity, although in-vivo subtype selectivity or differential pharmacokinetics for the M2 subtype has been suggested. One major disadvantage of the tracer is the low (<20%) radiochemical yield of the synthesis procedure. Other approaches to image muscarinic receptors non-selectively include the PET-tracers [N-11C-methyl]-benztropine, various analogues of [11C] labeled N-methyl-4-piperidyl-benzilate or tropanyl benzilate and...
[11C]scopolamine. Additionally, non-subtype selective imaging of muscarinic receptors has also been performed using [123I]iododextrimide SPECT.

Candidate tracers with subtype selectivity for the M2 receptor have also been synthesized. The most promising and best characterized of these tracers is [18F]FP-TZTP, a muscarinic agonist intended for use as a PET radiotracer. Numerous studies including rat, monkey and human studies have been performed using this tracer. Although selectivity for the M2 receptor as compared to the other muscarinic receptor subtypes was not evident in-vitro, preferential binding to M2 receptors was shown in-vivo in pharmacological blocking studies in muscarinic receptor knockout mice and the reason for this may be a slower dissociation of the tracer from M2 receptors. Human studies in normal controls showed a positive correlation with age and also higher tracer binding in humans with the ApoE4 genotype, which may be due to decreased competition by endogenous ACh as compared to young subjects or normal elderly, respectively.

Less promising, but well described radiotracer is [123I]Z-IQNP, a radiotracer that has a higher affinity for the muscarinic M2 receptor as compared to the M1 receptor, but has only shown to be of value for the evaluation of M2 receptors in the cerebellum. Other attempts to synthesize a radiotracer for the M2 receptor were based on the compound BIBN 99136, derived from AF-DX 116 which is an in-vitro tracer for the M2 receptor that does not penetrate the blood-brain-barrier, and [18F] radiofluorinated derivatives of QNB. So far, these tracers have shown either a lack of subtype selectivity or inappropriate tracer kinetics.

**Imaging of the nicotinic neurotransmitter system**

As for imaging of the muscarinic receptor system, in-vivo mapping of the nicotinic receptor system has been hampered by the lack or limited availability of appropriate radiotracers. Tracer delivery in the brain of [11C]nicotine has proved to be mainly determined by cerebral perfusion, blood-brain-barrier transport or non-specific uptake and did not necessarily correlate with nicotinic receptor availability. Recently, [18F], [11C] and [123I] labeled derivatives of A-85580, a pyridine, were evaluated as specific radiotracers for the nicotinic α4β2 subtype, and have shown promising results. Other approaches for imaging of the nicotinic neurotransmitter system included radiotracers that are based on epibatidine. One of these is [18F]NCFHEB, which shows a higher brain uptake, better correlation with nicotinic receptor density and...
faster kinetics than radiofluorinated A-85380 derivatives, and this particular tracer may be suitable for clinical use.

**Imaging of the glutamatergic system**

The alterations in the glutamatergic system in many neuropsychiatric diseases have led to the development of several candidate radiotracers that target in intra-channel PCP or MK-801 site, which include the PET tracers [18F]MK-801, [11C]ketamine, [18F]AFA, [11C]GMOM and the SPECT tracer [123I]CNS-1261. Although developments in this field are fast, no tracers that display high in-vivo specificity have been synthesized to date and human imaging studies using these tracers have shown that the tracers are either ineffective or not yet fully characterized.

**Amyloid imaging agents**

Another exciting recent development in the field of imaging of dementia are amyloid tracers, which have shown retention of tracers such as the thioflavine derivative [N-methyl-11C]Pittsburg Compound B (PIB) or [18F]FDDNP. Of these, the latter binds to both Aβ and NFTs, whereas the former only labels Aβ in plaques (but not soluble Aβ), which is considered an advantage as compared to concomitant binding to NFTs. Although interesting results have been obtained with these tracers, the in-vivo specificity of such tracers remains questionable, since up to 50% of elderly with mild cognitive impairment show high [N-methyl-11C]PIB binding in the brain. Other potential radiotracers that have been synthesized and evaluated in-vivo include [11C]SB-13, [11C]BF-227 and [11C]MeS-IMPY, [18F]-BAY94-9172, which only labels Aβ and has the practical advantage of being a 18F labeled tracer, which makes it more suitable for clinical application. Although the value of such tracers has yet to be established, and may be limited for early diagnosis of dementia, differentiation between AD and other causes of dementia such as dementia with Lewy bodies or frontotemporal dementia may be possible using these tracers.

**Aims and outline of the thesis**

The aims of the present thesis were to explore novel candidate radiotracers intended for imaging of dementia by means of molecular imaging techniques, and to evaluate the value of existing radiotracers that are currently being used, or could potentially be used for imaging of dementia. The thesis is subdivided in five parts. Part one (this part) includes a gen-
eral introduction on the epidemiology and neuropathology of various forms of dementia including the role of dysfunction of the cholinergic (and glutamate) neurotransmitter system in dementia. Also the present methods of imaging of dementia using nuclear medicine imaging techniques are described, as well as potential future appliances of PET or SPECT imaging in dementia. Part two of the thesis describes a series of in-vitro and related in-vivo experiments that were conducted using newly synthesized compounds intended as potential SPECT radiotracers for imaging of the muscarinic M2 receptor in cognitive disorders and dementia. Part three of the thesis contains in-vivo experiments on existing radiotracers that bind to receptors, with the focus on dementia and cognitive disorders. First, validation of an autoradiographic method, which is used in two other chapters in this thesis, for use with short-living radioisotopes as an alternative method for biodistribution experiments is described. Then the in-vivo binding characteristics of \([^{123}\text{I}]\text{CNS-1261}\), a potential radiotracer for activated NMDA receptors are reported. The next two chapters evaluate the influence of psychopharmaceuticals on the binding of previously developed radiotracers for use in dementia. Another chapter reports on the effects of disruption of the dopaminergic neurotransmitter system on the binding of the muscarinic receptor radiotracer \([^{123}\text{I}]\text{Iododexetimide}\) in the rat brain. In part 4 of the thesis, a study is presented that covers the differences in binding of \([^{123}\text{I}]\text{Iododexetimide}\) in patients suffering from PDD versus PD patients. In part five of the thesis, a brief summary of the thesis is given and general conclusions are drawn from the conducted experiments. A more detailed overview of the experimental sections of the thesis is outlined below.

**Chapter 2.** In this chapter, the synthesis of a series of iodinated TZTP-derivatives as potential radiotracers for imaging of muscarinic M2 receptors using SPECT is described. The compounds were tested in-vitro and the most promising compound was tested in-vivo in rats. This chapter also describes the metabolism of the compound that was tested in-vivo and the effects of different types of anesthesia on the outcome of the in-vivo study.

**Chapter 3.** Data of in-vitro competitive binding experiments using newly synthesized candidate tracers for muscarinic receptors are presented in this chapter. The chapter covers a series of synthesized 6β-ace-toxynortropane analogues, of which the parent compound was previously shown to have a very high affinity and selectivity for the muscarinic M2 receptor, and the synthesis of the compounds is described.
Chapter 4. Storage phosphor imaging may be an attractive alternative for biodistribution studies on short-living neuroreceptor radiotracers in small animals, and may provide more accurate measurements in small or complex brain areas as compared to the classical dissection technique. This chapter describes the experiments that were performed to validate the storage phosphor imaging technique for use with a series of short-living radioisotopes, such as $^{123}$I. Results of ex-vivo autoradiographic studies using $[^{123}I]$FP-CIT on rat brain sections are compared with data obtained from dissection experiments.

Chapter 5. Excessive activation of NMDA receptors may play a critical role in the neurodegenerative events in diseases such as AD or Lewy body disorders. Imaging of the NMDA receptor may help to diagnose patients that are suffering from neurodegenerative diseases, evaluate disease activity and may help to select patients that may respond to NMDA receptor inhibitors. In this chapter, the ex-vivo binding of the recently developed radiotracer $[^{123}I]$CNS-1261 to NMDA receptors in the rat brain is evaluated using the storage phosphor imaging technique. Specific binding of the radiotracer is demonstrated by blocking of the intra-channel binding site using MK801 and an attempt was made to increase binding by co-activation of the receptor by injection with D-serine.

Chapter 6. The dopamine transporter radiotracer DaTSCAN ($[^{123}I]$FP-CIT) has proven to be a valuable diagnostic tool to discriminate Lewy body dementia from Alzheimer’s dementia, and was recently approved for this indication. Many patients that are referred for such scans already use acetylcholinesterase inhibitors, but the effects of such medication on the binding of the radiotracer to dopamine transporters in the living brain are unknown. In this chapter, the effects of single intravenous administration and single or subchronic administration of the acetylcholinesterase inhibitors rivastigmine and donepezil on striatal $[^{123}I]$FP-CIT binding in rats are evaluated using the classical dissection technique.

Chapter 7. In-vivo muscarinic receptor SPECT, using non-selective muscarinic radiotracers such as $[^{123}I]$Iododextemide, may be of value to select cognitively compromised patients that could benefit from acetylcholinesterase inhibitors. However, many patients will already use neuroleptics when such imaging procedures will be performed. These neuroleptics may influence the binding of muscarinic receptor tracers in the brain. This chapter reports on the effects of repeated administration of $[^{123}I]$Iododextemide.
the neuroleptics olanzapine and risperidone on the biodistribution of $^{[123]}$Iododexetimide in the rat brain.

Chapter 8. Cholinergic disturbances attribute to the cognitive disturbances that are present in the Lewy body disorders PD, PDD and DLB. Disruption of the dopaminergic system is thought to induce hyperactivation of the cholinergic neurotransmitter system and this may be detected by in-vivo imaging using $^{[123]}$Iododexetimide as a radiotracer. In this chapter, the effects of a unilateral hypodopaminergic state of the brain, resulting from a unilateral 6-hydroxydopamine lesion (an animal model for PD) on the biodistribution of $^{[123]}$Iododexetimide are reported.

Chapter 9. Cognitive impairment in Parkinson’s disease (PD) and Parkinson’s disease related dementia (PDD) are associated with deficiencies in the cholinergic neurotransmitter system. In chapter 9, we present data from a human brain SPECT study that compares the distribution of $^{[123]}$Iododexetimide in PDD patients to the distribution in PD patients.

References


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