Fundamental studies on radiotracers intended for receptor imaging in dementia

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CHAPTER 10

SUMMARY

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. In this chapter, the studies that were performed to accomplish these aims, are summarized.

Chapter 2 describes the synthesis of a series of iodinated TZTP-derivatives as potential radiotracers for imaging of muscarinic M₂ receptors. Such tracers would allow visualization and quantification of the presynaptic muscarinic receptor system by means of SPECT or PET and may be of value to monitor disease progression and effectiveness of experimental therapeutic interventions in neuropsychiatric diseases characterized by a central cholinergic deficit, e.g., Alzheimer’s disease (AD). In the reported study, derivatives of 5-[(4-alkylthio)-1,2,5-thiadiazol-3-yl]-1,2,3,6-tetrahydro-1-methylpyridines (TZTPs), originally described by Sauerberg¹ and proposed as potential tracers for muscarinic receptors²,³, were synthesized and tested for affinity to cloned human muscarinic receptor subtypes by in-vitro competitive binding assays. Several of these compounds showed a high affinity for these receptors, within the nanomolar range. The most promising of the novel potential tracers, the iodinated 5-(E)-iodopentenylthio-TZTP, showed a high affinity to M₂ receptors (4.9nM) and favorable selectivity ratios over M₁ and M₃ receptors, was radioiodinated and subsequently subjected to in-vivo biodistribution and blocking experiments in rats. Also, the metabolism of this derivative was studied by TLC. Distribution of the potential tracer in the rat brain occurred in a M₂-like pattern but blocking effects of the potent muscarinic antagonist scopolamine were only detected until 15 min after injection of the potential tracer, and thus specific in-vivo binding could not be demonstrated after that time point. The value of the new tracer proved to be limited by high non-specific uptake of the original tracer due to high lipophilicity and rapid metabolism of the compound.
Chapter 3 discusses another series of in-vitro competition studies, using newly synthesized compounds based on 6β-acetoxynortropane, a tropane alkaloid that was reported earlier to have high affinity and selectivity for the muscarinic M2 receptor subtype as compared to the other muscarinic receptor subtypes. Due to the nature of the molecular tropane skeleton, many derivatives, such as [123I]/[18F]FP-CIT or [123I]β-CIT, have proven to be useful as radiotracers for receptor imaging in the living human brain. The challenge of this experiment was to create a derivative of 6β-acetoxynortropane suitable for radioiodination, while preserving the affinity for the M2 receptor. Moreover, we also attempted to optimize lipophilicity as compared to the TZTP-derivatives that are described in chapter 2, and to improve metabolic stability while maintaining the size of the molecule as small as possible. Four analogues of the molecule were synthesized, each containing an iodophenyl or phenyl moiety that could potentially be radioiodinated for use as radiotracers for imaging of the muscarinic M2 receptor subtype in neurodegenerative or neuropsychiatric diseases. As in chapter 2, the affinity of these derivates for muscarinic receptor subtypes was assessed in-vitro by competitive binding assays on muscarinic receptors. Unfortunately, substitution of an iodophenyl or phenyl moiety on the original compound, severely reduced both the affinity and selectivity of the derivatives for the muscarinic M2 receptor subtype and therefore it was concluded that the synthesized analogues were not suitable for use in human SPECT imaging.

In chapter 4, an alternative method for biodistribution experiments in small animals, which is used in chapters 5 and 8 of this thesis, is validated. The conventional dissection technique that is described in chapter 2 may not be adequate to study the biodistribution of radiotracers in small or complex brain structures in small laboratory animals such as rats or mice. Autoradiography using X-ray film allows much more precise measurements, but does not show a linear response to the amount of radioactivity that is exposed to the film. Moreover, X-ray film autoradiography requires long exposure times and therefore it is not suitable for imaging of short-living radioisotopes such as 123I. Storage phosphor imaging plates are used extensively in digital X-ray diagnostics and also increasingly for autoradiography in radiotracer biodistribution studies, although the response of these plates to short-living radioisotopes is unknown. In chapter 4, the properties of the imaging plates are compared directly for 11 short-living radioisotopes (18F, 32P,
Linearity, sensitivity, efficiency and resolution were measured for all isotopes. A linear response over a wide range of applied radioactivity was demonstrated for all tested isotopes. The plates showed the highest efficiency and sensitivity for strong $\beta^-$-emitters and the $\beta^+$-emitter $^{18}$F, whereas lower efficiency and sensitivity was detected for $\gamma$-emitters. For the $\gamma$-emitters, only low energy $\gamma$-radiation seemed to interact substantially with the plate. The resolution of the images that could be obtained proved to be worse for high energy $\beta$-emitters whereas the best resolutions were obtained with low energy $\gamma$-emitters such as $^{125}$I (0.32mm full width at half maximum; FWHM). Also an ex-vivo study is presented in chapter 4, in which the biodistribution of the dopamine transporter tracer $[^{123}$I]FP-CIT was studied in the rat brain, with and without blocking of the receptor by pre-administration of methylphenidate, using either the conventional dissection technique or the storage phosphor imaging technique. The results of both studies proved to be comparable but the results that were obtained from the imaging plates showed less variability. It is concluded from chapter 4 that, although the properties of the imaging plates vary considerably for various radioisotopes, the storage phosphor imaging technique is a very attractive alternative to conventional dissection studies when using short-living radioisotopes such as $^{125}$I.

Chapter 5 describes the appliance of storage phosphor imaging in an experiment that assessed the ex-vivo binding in the rat brain of a recently proposed radiiodinated tracer for SPECT imaging of activated NMDA receptors, $[^{123}$I]CNS-1261. Excessive activation of NMDA receptors, the most abundant receptor of the glutamatergic neurotransmitter system, is believed to contribute to neuronal death in neurodegenerative diseases such as AD or Lewy body disorders. Inhibition of NMDA receptor activation by substances such as memantine, may have neuroprotective effects and (semi)quantitative imaging of the activated system may be useful to select patients for such inhibition therapies. Chapter 5 first reports on a time course dissection biodistribution experiment in rats, which determined the time point at 2h after injection as optimal for studying of the tracer distribution in the brain. Second, an experiment is described in which the distribution in the rat brain was studied using storage phosphor imaging in control animals versus animals that were pretreated with either D-serine alone or D-Serine+MK801. D-serine is a substance that co-activates the NMDA receptor and pre-administration should therefore hypothetically lead to a higher binding of $[^{123}$I]CNS-
MK801 blocks the binding of the radiotracer to its binding site and proved to decrease the uptake of the radiotracer in brain areas that express relatively high densities of NMDA receptors. Although this decrease was significant as compared to control rats, strongly suggesting specific binding of the tracer, the observed reduction of binding was small and this is also very suggestive of a high degree of non-specific uptake of the tracer. Thus, our study has shown that \([^{123}\text{I}]\text{CNS-1261}\) binding is influenced by NMDA receptor availability. However, high non-specific uptake may limit quantification and small changes in receptor availability are unlikely to be detected and therefore its use in human SPECT imaging of activated NMDA receptors may be limited.

Another radiotracer that has proven to be of value for SPECT imaging in dementia is \([^{123}\text{I}]\text{FP-CIT}\). This dopaminergic transporter tracer, originally developed for the detection of degeneration of the nigrostriatal projection in Parkinson’s disease (PD), was registered recently to differentiate AD from dementia with Lewy bodies (DLB) by means of brain SPECT imaging. DLB is the second most common form of dementia after AD, and is often clinically misinterpreted as AD. In contrast with AD, DLB is characterized by pronounced dopaminergic cell loss\(^{10}\), and can thus be differentiated from AD by \([^{123}\text{I}]\text{FP-CIT}\) SPECT. In chapter 6, the effects of AChEIs on the binding of \([^{123}\text{I}]\text{FP-CIT}\) to dopamine transporters are reported. Apart from dopaminergic deficits, there is also a cholinergic deficit in DLB and cognition of such patients is improved by early AChEI therapy\(^{11}\). Since many patients that are referred for \([^{123}\text{I}]\text{FP-CIT}\) brain SPECTs already use medication of this type, and since cholinergic medication may influence the dopamine transporter availability, it is of considerable interest to know the influences of AChEIs on the \([^{123}\text{I}]\text{FP-CIT}\) binding in the brain.

In chapter 6, data is presented from a dissection experiment in which rats were treated with the AChEIs rivastigmine or donepezil or the potent dopamine transporter antagonist methylphenidate. The animals were treated either once intravenously or once orally shortly before injection of the tracer, or during 14 consecutive days (subchronic group) before injection of the tracer. As expected, the methylphenidate group showed a significant decrease in \([^{123}\text{I}]\text{FP-CIT}\) binding in the rat brain at 2h after injection of the tracer, after both intravenous and oral pre-administration shortly before injection of the tracer, but not after sub-chronic administration of the tracer. Neither acute nor subchronic pre-
administration of both AChEIs influenced the binding of the tracer in the rat brain. It is concluded that in rats, administration of the tested AChEIs does not lead to an important alteration of the dopamine transporter availability in the brain. Therefore, it is unlikely that AChEIs will induce large effects on the interpretation of $[^{123}]$FP-CIT SPECT scans in humans.

Chapter 7 also deals with the effects of psychopharmaceuticals on the binding of a neuroreceptor radiotracer. Similar to the experiment that is discussed in chapter 6, the experiment that is described in this chapter addresses the influences of repeated administration of neuroleptics on the binding of $[^{123}]$Iododexetimide in the rat brain. $[^{123}]$Iododexetimide was previously developed and evaluated as a non-subtype selective muscarinic receptor SPECT tracer$^{12,13}$, and would therefore perform less well as compared to muscarinic receptor subtype selective radiotracers, if these would be available. However, since a marked overall decrease of muscarinic receptor density has been shown in AD, DLB and PDD$^{14,15}$, this tracer may be of interest to study the integrity of the total muscarinic system in such patients (see chapter 9 for a human study using this tracer). Unfortunately, many of these patients use neuroleptics, which primarily target the dopaminergic system but which may (in)directly influence the cholinergic system, and therefore it is important to know the effects of these types of medication on the binding of $[^{123}]$Iododexetimide in the brain. In chapter 7, an animal experiment is presented that evaluates potential influences of repeated administration during 14 consecutive days of the atypical neuroleptics olanzapine and risperidone on the binding of $[^{123}]$Iododexetimide in the rat brain. Tracer uptake in muscarinic receptor-rich brain areas (i.e. cortical areas and striatum) and in brain areas expressing relatively low levels of muscarinic receptors (i.e. hypothalamus) was compared to data obtained from control rats. Since we were particularly interested in subchronic effects, radiotracer uptake was evaluated after one day of washout of the neuroleptics. No influences on tracer binding were detected after treatment with olanzapine. In the risperidone group, only an unexpected increase in tracer binding was found in the hypothalamus as compared to the control group. Since in-vivo studies in humans can only accurately assess muscarinic binding in muscarinic-rich brains areas, the data suggest no large non-acute effects of olanzapine and risperidone on muscarinic receptor imaging in humans, when using $[^{123}]$Iododexetimide.
Chapter 8 further reports on the association between the dopaminergic and the cholinergic system. In this chapter, data are presented from an animal experiment in which rats were given a unilateral selective dopaminergic brain lesion, and the effects of this lesion on the distribution of $[^{123}\text{I}]$Iododexetimide in the rat brain are revealed. The dysfunctional cholinergic neurotransmitter system in PD, PDD and DLB is thought to contribute to the cognitive deficits in these diseases. Besides degeneration of the cholinergic system, dopaminergic disruption which also occurs in these diseases, may directly influence the function of the cholinergic system. In-vivo imaging of the cholinergic system in such patients may be of value to monitor central cholinergic disturbances and to select cases in which treatment with cholinesterase inhibitors could be beneficial. To study the effects of a selective dopaminergic lesion on the $[^{123}\text{I}]$Iododexetimide binding, a group of rats was given a unilateral 6-hydroxydopamine lesion, and the damage to the dopaminergic system was confirmed by small animal SPECT imaging using $[^{123}\text{I}]$FP-CIT. Also, the effects of the lesion on the brain perfusion were measured using SPECT imaging and a significantly lower perfusion was detected in the striatum on the side of the lesion but not in the ipsilateral cerebral cortex. Then, the effects of the lesion on the binding of $[^{123}\text{I}]$Iododexetimide in a series of brain structures was measured using the storage phosphor imaging technique that is described in detail in chapter 4. The phosphor imaging data revealed a consistent and statistically significant lower tracer binding in all examined neocortical areas on the ipsilateral side of the lesion as compared to the contralateral side. In the hippocampus and subcortical areas such as the striatum, such asymmetry was not detected. The data that are presented in this chapter suggest that evaluation of the muscarinic receptor availability in the dopamine depleted brain, using $[^{123}\text{I}]$Iododexetimide is feasible, and also that a 6-hydroxydopamine lesion induces a decrease of neocortical muscarinic receptor availability. This may be due to downregulation of postsynaptic muscarinic M₁ receptors, or direct competition of acetylcholine as a result of hyperactivation of the cortical cholinergic system in response to the disruption of the dopaminergic pathway.

Chapter 9 presents a human $[^{123}\text{I}]$Iododexetimide SPECT study that was performed in PD and PDD patients. Since $[^{123}\text{I}]$Iododexetimide SPECT may help to select cognitively compromised patients that could benefit from AChEIs or to monitor disease progression, this study was conducted to investigate the differences in muscarinic receptor avail-
ability in the brain of PDD versus PD patients, which was measured in-vivo. The SPECT studies were performed in 13 subjects (6 PD, 7 PDD) and the obtained brain SPECTs were fitted to a standard brain template, normalized to non-specific tracer uptake in white matter and analyzed using statistical parametric mapping (SPM99). No increases in tracer binding were detected in the brain of PDD versus PD patients. However, significant decreases in tracer binding in the PDD group as compared to the PD group were detected in both the left and right temporal cortex, left hippocampus, and the posterior cingulate cortex. The data that are presented in chapter 9 demonstrate decreased muscarinic receptor binding by $^{[123I]}$Iododexetimide in PDD versus PD in brain areas that are involved in memory function. The findings suggest that reduced muscarinic receptor binding in these brain areas could be used as a marker for PDD. Additionally, in-vivo $^{[123I]}$Iododexetimide SPECT-imaging might be of value for the selection of PDD patients that are likely to respond to AChEIs.

References


