SUMMARY AND DISCUSSION

The aims of the studies described in this thesis were (1) to develop methods for quantifying P-glycoprotein (Pgp) function at the blood-brain barrier (BBB) using the Pgp substrate tracer (*R*)-[^{11}C]verapamil and positron emission tomography (PET), and (2) to evaluate BBB Pgp function in healthy aging and AD. These studies are important as they may provide further insight into the role of BBB Pgp function in both normal aging and the pathophysiology of AD.

To achieve these aims, several steps were performed. First, several methodological issues were investigated. Various tracer kinetic models for analyzing (*R*)-[^{11}C]verapamil data were developed and the reproducibility of these models was assessed. Next, (*R*)-[^{11}C]verapamil PET studies were performed in a relatively large group of healthy subjects, both males and females, in different age groups to investigate the effect of aging on BBB Pgp function. Effects of gender on BBB Pgp function were also assessed. Next, BBB Pgp function was investigated in AD patients and compared with healthy elderly subjects. In addition, (*R*)-[^{11}C] verapamil PET studies were performed in AD patients with and without characteristics of cerebral amyloid angiopathy (CAA), in order to evaluate the effect of CAA on Pgp function. Finally, effects of genetic variations in the Pgp-encoding ABCB1 gene on BBB Pgp function were assessed in both healthy subjects and patients with Alzheimer's disease.

In this chapter the main findings of these studies are briefly summarized and discussed. In addition, possibilities and directions for future research are discussed.

Main findings

First, methodological aspects of (*R*)-[^{11}C]verapamil PET studies were assessed. In **chapter 2**, test-retest (TRT) variability of (*R*)-[^{11}C]verapamil PET studies was evaluated for several tracer kinetic models used for analysis of (*R*)-[^{11}C]verapamil data [1-3]. In addition, the impact of corrections for partial volume effects (PVE) on reproducibility was assessed. All data were analyzed using single-tissue and two-tissue compartment models, and global and regional TRT variability was determined for various parameters and outcome measures. Analysis using the Akaike information criterion showed that a constrained two-tissue compartment model provided the best fits to the data. Global TRT variability of the volume of distribution (V_T) was comparable for single-tissue (6%) and constrained two-tissue (9%) compartment models. TRT variability of binding potential (BP_{ND}) derived from the constrained two-tissue compartment model was less robust, but still acceptable (22%). After applying PVE correction, there was essentially no change in TRT variability. It was concluded that the model of choice for analysing (*R*)-[^{11}C]verapamil data is a constrained two-tissue compartment model.

Next, in **chapter 3**, effects of age and gender on BBB Pgp function were assessed. Age is a risk factor for many neurodegenerative disorders, such as AD and Parkinsons's disease [4-5]. Loss of Pgp function with increasing age may be involved in the development of

those disorders. This may differ between males and females as, for AD, female gender is another risk factor [6-7]. Thirty-five healthy men and women in three different age groups were included and (*R*)-[^{11}C]verapamil PET data were obtained. In older men, increased V_T of (*R*)-[^{11}C]verapamil was found in several large brain regions, suggesting a decrease in Pgp function with age in men. Young and elderly women, however, showed comparable V_T values, suggesting no effect of age on BBB Pgp function in women. When compared with young men, young women had higher V_T values. Data in this study were assessed with and without PVE correction, again showing comparable results in V_T values. In conclusion, decreased BBB Pgp was found with aging, but effects of age on BBB Pgp function differed between men and women.

In **chapter 4**, BBB Pgp function in AD patients was compared with healthy age-matched control subjects. A major pathological hallmark of AD is the accumulation of amyloid-beta (Aβ) in the brain, which can be visualized using PET and the amyloid ligand [^{11}C]PIB [8-9]. In thirteen [^{11}C]PIB positive AD patients global (*R*)-[^{11}C]verapamil BP_{ND} values were increased significantly compared with fourteen healthy controls. Higher (*R*)-[^{11}C]verapamil BP_{ND} values were also found in AD for frontal, parietal, temporal and occipital cortices, and for posterior and anterior cingulate, suggestive of decreased BBB Pgp function in these regions. No differences were found in medial temporal lobe and cerebellum. No significant correlations were found between BP_{ND} of (*R*)-[^{11}C]verapamil and [^{11}C]PIB BP_{ND}, which may be due to a ceiling effect of amyloid pathology in AD patients. Nevertheless, findings indicate that Pgp function is decreased in AD patients, supporting the hypothesis that decreased Pgp function contributes to Aβ accumulation and may be involved in the pathogenesis of AD.

Next, as decreased BBB Pgp function was shown in AD patients using (*R*)-[^{11}C]verapamil PET, we investigated in **chapter 5**, eighteen [^{11}C]PIB positive AD patients, of which six had microbleeds (MBs) in the brain. MBs are thought to be an indication of CAA, a condition in which Aβ accumulates on brain blood vessel walls [10]. CAA is often found in AD brains, though with varying degree of severity [11-12]. Pgp dysfunction is thought to promote CAA development [13]. In this pilot study we found no differences in BP_{ND} of (*R*)-[^{11}C]verapamil between these relatively small patient groups, suggesting no evidence for additional Pgp dysfunction in AD patients with MBs.

Finally, in **chapter 6,** the effects of genetic variations in the highly polymorphic Pgp-encoding ABCB1 gene [14-15] on BBB Pgp function were assessed, both in healthy subjects and in AD patients. Three common single nucleotide polymorphisms (SNPs) were tested (C1236T, C2677A/T, C3435T) and correlated with BBB Pgp function as measured with (*R*)-[^{11}C]verapamil PET. In healthy subjects no effects of SNPs were found. In contrast, in AD patients with one or more T present in C1236T, G2677T and C3435T, (*R*)-[^{11}C]verapamil BP_{ND} was found to be significantly higher when compared to patients without a T. An effect of T dose in C1236T and G2677T on BP_{ND} was found, with higher BP_{ND} values as T dose increases, suggesting decreased BBB Pgp function in AD patients with these genetic variants.