The link between ceramide transporters, innate immunity and Alzheimer's disease

SUMMARY

Alzheimer's disease (AD) is an enormous and increasing burden on society in many developed countries. Despite intense research efforts, there is still no effective cure. This indicates that we should direct more of our research effort to understanding the basic pathological processes of this disease and methods of drug delivery. It has recently emerged that sphinglolipids, and in particular ceramides, are involved in the pathological processes of AD, but the exact mechanisms are currently unknown. The research described in this thesis is focused on two topics: The first topic is ceramide transporters and inflammation in neurodegenerative disease (Chapter 1-3), the second is nanoparticles for delivery of drugs to the brain (Chapters 4 and 5).

In **Chapter 1** we investigated the interaction between ceramide transporters (CERT) and Serum Amyloid P component (SAP). SAP is a non-fibrillar glycoprotein belonging to the pentraxin family of the innate immune system. We demonstrated that CERT specifically binds serum amyloid P (SAP) in its physiological conformations, pentamers and decamers. The START domain in CERT is important for this interaction. SAP and CERT form complexes in blood and partly colocalize in amyloid plaques from AD patients.

Additionally, we report a novel function of CERT/GPBP in complement activation in **Chapter 2**. Both CERT isoforms were found to bind the globularhead region of C1q and to initiate the classical complement pathway. In addition, C1q was shown to bind to endogenous CERT on the surface of apoptotic cells. These results demonstrate the involvement of CERTs in innate immunity, especially in the clearance of apoptotic cells.

In **Chapter 3** we used design-based stereology to quantify the number of CERT immunoreactive cells in the striatum of 6-hydroxydopamine (6-

OHDA) lesioned rats as a model of Parkinson's disease (PD). No difference in the striatal expression levels of CERT/GPBP proteins was found between diseased and control animals, suggesting that the expression pattern of CERT in the striatum is not affected in the 6-OHDA rat model of PD.

Chapter 4 describes development of an enzyme linked immunosorbent assay (ELISA) for the detection of peptides functionalized with biotin and fluorescein groups. We were able to accurately measure peptides bound to pentafluorophenyl methacrylate nanoparticles in blood plasma of rats after injection, with a detection limit of 1 ng/mL. This novel method is a valuable tool in determining the effectiveness of nanoparticle targeting.

In **Chapter 5** we evaluated the efficacy of delivery and safety of liposome-, polyester-, poly(glycidol)- and acrylamide-based nanoparticles functionalized with peptides targeting brain endothelial receptors, *in vitro* and *in vivo*. We detected labelled nanoparticles in brain homogenate, liver homogenate, in cerebrospinal fluid and blood plasma after injection. Based on both the *in vitro* and *in vivo* results, we concluded that the physical characteristics of the nanoparticles and the targeting peptides used need to allow for preferential binding to brain endothelial cells compared to endothelial cells of other tissues for efficient blood-brain barrier transport.