Cholinergic deficiency and inflammation in cognitive dysfunction
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Chapter 9

General discussion
In this concluding chapter the main findings of this thesis will be summarized with reference to the various relevant theoretical frameworks. After some introductory remarks on the relation between impairments of cognitive functioning in relation to neurodegenerative disease and specific neurotransmitter systems, the focus will be on the cholinergic system and on inflammatory mechanisms, and on the interplay between these two. Based on this discussion, the chapter concludes with specific suggestions for further research.

Cognitive functioning depends on the interplay of many parts of the brain. Disruption of the cognitive functions can be caused by different disease mechanisms affecting these areas. The most tragic is probably cognitive decline due to degenerative processes. Much research focuses on the etiology, pathological mechanisms and genetic background of these diseases. The past two decades in which molecular biology flourished, brought insight into specific pathological processes of the various diseases that cause dementia. It is now well established that Alzheimer’s disease (AD), the most common form of dementia, is characterized by accumulation of amyloid-beta into amyloid plaques in the brain parenchyma and the formation of intraneuronal tangles as a result of abnormal phosphorylation of the protein tau. These plaques and tangles are accompanied by a marked loss of neurons in the neocortex and hippocampal formation. The major component of Lewy bodies, the pathological hallmark of Dementia with Lewy bodies (DLB) and Parkinson’s disease (PD), is aggregated alpha-synuclein. Lewy bodies and Lewy neurites are found both in the brainstem (substantia nigra) as well as throughout the cortex of patients suffering from these diseases. This general pattern of protein-misfolding that leads to intra- or extracellular aggregates in the brain has been found in other disease associated with dementia, frontotemporal dementia (tau) and prion-disease among others. Specific symptoms in patients suffering from neurodegenerative disease can be partly explained by the localization of the specific disease processes in the brain. The abundant plaques and tangles in the hippocampus in AD can account for the profound memory complaints in this disease. The affected neurons in the substantia nigra in alpha-synucleinopathies explain the syndrome of parkinsonism. The current understanding of these disease-mechanisms has now fuelled extensive research into the development of drugs that target the abnormal processing of the protein involved, thus preventing aggregation and accumulation of these proteins in the brain. Up till now no such treatment is available to stop degeneration of brain tissue for any of the conditions mentioned above. Although pathological changes of specific proteins are the basis of cognitive decline in neurodegenerative disease, other pathological processes that accom-
pany these diseases can account for specific symptoms. Some of these processes can be targeted by current available medication. Disturbances in various neurotransmitter systems have been described in neurodegenerative diseases. Of these, the cholinergic system has been most widely studied. The development of cholinesterase inhibitors was based on the evidence of cholinergic dysfunction in AD. Inflammation in the brain is commonly seen in degenerative processes. Although this could be beneficial as well as detrimental to the pathological changes, it possibly affects the symptoms observed in these diseases. Anti-inflammatory agents could counteract on these processes. In this thesis we focused on these two phenomena, cholinergic dysfunction and neuroinflammation, that are both known to play an important role in neurodegenerative diseases causing dementia. The choice for this combination was not arbitrary. Cholinergic dysfunction seems to comprise a set of symptoms which overlap with delirium. Delirium can be elicited both by medication with anticholinergic side effects as well as by infectious disease. Recent findings from experimental studies showed that the cholinergic system and the innate brain immune system influence each other. So the cholinergic system and innate immune system of the brain seem to act together in pathological processes, they contribute both to specific symptoms, and they can be influenced by medication that is readily available.

THE CHOLINERGIC SYSTEM

The cholinergic transmission is one of the most important modulating systems in the brain. The origin of this projection is located in the magnocellular neurons of the basal forebrain, the nucleus basalis of Meynert and the substantia innominata. These extensive cholinergic projections influence nearly all aspects of cognitive functions, especially the domains of attention, memory and emotion. Neurochemical reductions in cholinergic enzymes and/or loss of cholinergic neurons are apparent in many brain disorders associated with neuropsychiatric symptoms like AD, DLB, PD, progressive supranuclear palsy and Down’s syndrome. In Chapter 2 we hypothesised that loss of cholinergic neurons can lead to a clinically identifiable syndrome. However, to date there is no clear description of the clinical picture in patients suffering from cholinergic deficiency. Based on (1) data on the effects of anticholinergic drugs, (2) case studies of patients receiving CEI therapy and on (3) experimental work relevant to the functional ramifications of cholinergic neurotransmission, we formulated in this chapter a hypothesis on the clinical characteristics that may result as the consequence of cholinergic deficiency: the ‘cholinergic deficiency syndrome’ (CDS). This syndrome is hypothesized to comprise impairments of attention and concen-
tration, anxiety, restlessness and hallucinations. Cognitive deficits may be secondary to the CDS since the cholinergically mediated fundamental functions are necessary for optimal performance of the cortical instrumental functions\textsuperscript{20,21}. It was proposed that the CDS could be a more rational indication for treatment with CEIs in patients with dementia than either a nosological entity or subgroups of patients characterised by an arbitrarily defined range of MMSE scores.

The study described in Chapter 3 was based on this hypothesis. Several clinical and neuropsychological parameters were investigated for their relationship with a beneficial response to the cholinesterase inhibitor rivastigmine. Our results showed that fluctuations in reaction time tasks and poor sustained attention at baseline are features that are associated with a favourable response to treatment with rivastigmine. Moreover, a cluster of behavioural symptoms, including hallucinations, apathy, anxiety and psychomotor disturbances, seems to coexists with these attentional deficits in patients that respond well to CEI therapy. Patients with clinical symptoms that respond well to CEI therapy, can be considered to have suffered from significant cholinergic deficiency before starting treatment. This exploratory study agrees with our hypothesis described in Chapter 2 that loss of cholinergic neurons is accompanied by a clinical syndrome as outlined above. This syndrome resembles the features seen in acute delirium. Future studies will have to clarify to what extent the CDS and delirium overlap and if CEIs may be effective also in the latter condition.

The clinical importance of recognising cholinergic deficiency is illustrated in Chapter 5. Patients with pathologically confirmed DLB were mistaken to suffer from Creutzfeldt Jakob disease due to a sensitivity reaction to neuroleptics. Neuroleptic sensitivity, defined as rigidity, sedation, increased confusion and sometimes pyrexia starting after administration of neuroleptic drugs, is a supportive feature of DLB and it is associated with increased morbidity and mortality\textsuperscript{22}. DLB is characterised by a more extensive loss of cholinergic neurons compared to other dementias, especially AD\textsuperscript{23}. In addition, in DLB the dopaminergic pathways are afflicted due to alpha-synuclein pathology in the substantia nigra and striatum. The exact mechanism of neuroleptic sensitivity is largely unknown. Early pathological studies revealed reduced upregulation of D2-receptors in DLB-patients with neuroleptic sensitivity\textsuperscript{24}. A dopamine/cholinergic dysbalance could contribute to the severe adverse reaction patients with DLB experience when (classical) neuroleptics are administered. Most neuroleptics have anticholinergic properties which can directly influence behavioral disturbances in cholinergic deficient patients\textsuperscript{25}. Neuroleptics are widely used to treat psychiatric disturbances in elderly patients with dementia. Prescription rates of more than 50% have been
reported. Acknowledgment of the CDS could prevent potentially dangerous side-effects in these patients due to these agents.

The relatively small study that we reported in Chapter 3 has limited value in defining the CDS-profile in very specific terms but gives a hint of the direction that can be taken to explore new ways of administering CEIs in a group of patients suffering from very disturbing symptoms. Independent prospective studies in larger cohorts of patients, with various neurodegenerative diseases should be performed to determine the true value of this or similar risk profiles. Ideally, this should converge to a specific clinical profile in patients with cholinergic deficiency, which can be easily recognised by a small set of simple tests in the physician’s office. An illustration of this line of reasoning is displayed in Box 1.

In theory other ancillary investigations could prove to be helpful in future to establish cholinergic deficiency, thus helping to predict a favourable response to CEIs. We investigated the possibility of quantitative spectral electroencephalography (qEEG) in this matter (Chapter 4). As in other studies, the qEEG showed an improvement in alpha rhythm after treatment with CEIs\textsuperscript{26,27}. However, in our population qEEG did not add to clinical measures in predicting response to CEIs. Possibly inter-individual physiological differences and cortical changes due to other pathological processes than cholinergic dysfunction hamper the use of qEEG as an objective measure of cholinergic deficiency.

Other biological markers such as cholinergic enzymes or anticholinergic activity measured in cerebrospinal fluid (CSF) could potentially be helpful in quantifying cholinergic deficiency. Few studies that address this issue have not shown satisfactory results yet. Levels of cholinesterases in serum and CSF have been measured in experimental settings. There was a dose-dependent change in inhibition of these enzymes when CEIs were administered, but no correlation with clinical symptoms have been described\textsuperscript{28,29}.

Positron emission tomography is an imaging technique that provides means to study neurochemical processes in vivo. Ligands have been developed to visualise the cholinergic system in the brain in patients with neurodegenerative disease\textsuperscript{30,31}. It is not possible yet to quantify cholinergic deficit by this method. Correlations with cognitive functions have been described but more extensive research need to be performed in order to establish the value of these techniques in clinical practice.

In conclusion, loss of cholinergic neurons due to underlying pathological processes can lead to a syndrome which can be clinically identified.
This profile, based on a combination of a few behavioural symptoms and simple neuropsychological tests that focus on attention, could potentially contribute to a better selection of patients that will experience unequivocal benefit from treatment with CEIs.

**Box 1. Cholinergic deficiency score as indicator for CEI-treatment**

Based on the findings of the study described in Chapter 3 we can assume that the cholinergic deficiency syndrome consist of attentional deficits and behavioural symptoms. This could be used to define a cholinergic deficiency score that can be expressed by a number. We took the three significant baseline variables (reaction time variability, sustained attention and the cluster of behavioural symptoms calculated by the NPI-subset) of all patients who participated in the study and divided the outcomes per variable into tertiles. Each tertile was given a score where lower tertiles were scored 0 and upper tertiles scored 2. Per patient a total score was calculated which could range from zero to six. Based on these total scores three separate groups are defined reflecting the probability of cholinergic deficiency: Group 1= low probability of cholinergic deficiency, total score 0-2 (n=16); Group 2= intermediate probability, total score 3-4 (n=9); Group 3 = high probability, total score 5-6 (n=9). This cholinergic deficiency score was plotted against the therapeutic response as indicated by the mean Zsum-score: the mean z-score per patient of the change scores on MMSE, IDDD and NPI (see methods section, Chapter 3). Although the use of the data of the present study for this purpose represents a circular line of reasoning to a certain degree, the figure illustrates that a composite measure of relevant clinical features believed to reflect baseline cholinergic deficiency, may be used to predict beneficial response to CEI treatment.

**Cholinergic deficiency scores and response to CEI-treatment**

Cholinergic deficiency score based on significant baseline variables (VRT-sd, CPT & NPI-cluster). Horizontal lines indicate mean Zsum score: the mean z-score per patient of the change scores on MMSE, IDDD and NPI, as an overall measure of therapeutic response.
INFLAMMATORY MECHANISMS

Chronic neurodegenerative diseases are known to be accompanied by immunological changes in the brain. This has been studied most extensively in AD, but it has also been shown in relation with Lewy body pathology, prion disease and amyotrophic lateral sclerosis. Microglia as the macrophages of the brain are central in this inflammatory reaction. In the normal brain microglia are characterized by a highly branched morphology and down-regulated phenotype. Any disturbance in their environment causes microglia to change their morphology, express all kind of antigens on the cell-surface and release cytokines. This is referred to as activated microglia. It is has been proposed that microglia go through different stages of activation with accompanying morphological and functional changes. Animal models of neurodegenerative diseases show an increase in activated microglia in the surroundings of pathological lesions. It remains to be elucidated whether this inflammatory response contributes to pathology in neurodegenerative diseases but it is clear that once initiated these inflammatory mechanisms affect the diseased brain. Microglia are also susceptible to changes in the peripheral immune system. Although once considered impossible due to the blood-brain barrier, animal studies in which LPS was administrated as a model for sepsis, revealed microglia activation and cytokine release in cerebral tissue, thus showing the ability of the central nervous system to induce an innate immune response when triggered by blood-borne pathogens. Our study described in Chapter 8 provided the first evidence of an immunological response, i.e. activated microglia, in human brain tissue related to a systemic inflammatory reaction. This response was found in the absence of other brain pathology. Our findings concur with the evidence from experimental studies that the brain actively participates in systemic infections.

Circulating cytokines in a systemic inflammatory response seem to be able to signal the brain and modify the cytokine profile within specific CNS regions. A heightened neuroinflammatory response and a modified cytokine milieu in the brain may lead to neurobehavioral impairments and even delirium. These consequences are even more distinct in animals with certain pre-existing conditions such as high age or chronic neurodegeneration in which microglia are already "primed". Several authors suggested the concept of 'primed' microglia. Aging itself is accompanied by a state of low-grade inflammation. The exact cause and meaning of this phenomenon remains to be elucidated but it is clear that this process is not limited to the systemic immune system but also afflicts the innate immune system of the brain. Due to the altered immune status caused by ageing, resting microglia change to a mildly activated state in old age. This is confirmed by our findings that in the relatively old population we studied in
Chapter 8 we found some level of microglial activation in both groups. This condition is enhanced by concomitant neurodegenerative disease (see above). Different experimental studies have shown that release of pro- and anti-inflammatory cytokines is under strict control in neurodegenerative disease and increase of cytokine-levels in the brain is modest (reviewed by Perry et al\textsuperscript{32}). During systemic infection or other pro-inflammatory insults such as e.g. an operation, these microglia that are already ‘primed’ become highly activated. This causes behavioral changes resulting in delirium, enhance cognitive symptoms in patients with degenerative brain disease and probably induce progression of this disease\textsuperscript{32,44,46,47}. It is tempting to speculate that similar mechanisms may play a role in humans, since both old age and neurodegenerative diseases predispose for delirium.

Previous studies showed associations between delirium and inflammatory serum-markers\textsuperscript{48-53} In Chapter 7 we studied the peripheral cytokine-profiles of elderly patients who underwent hip-surgery, a putative risk factor for the development of delirium. Our study was carried out in a relatively healthy population so confounding by e.g. acute illness, was minimized. We found no differences between patients who developed a delirium and those who did not. This means that it is not likely that cytokine-levels are risk factors for the development of delirium by themselves. Our results strengthen the idea that in order to develop behavioral changes due to an insult the brain should already be primed. The extent of change in the peripheral inflammatory status is not decisive for the risk of developing delirium. The presence and activation of already primed microglia due to aging and/or neurodegenerative changes probably determines the threshold for behavioral changes and delirium.

In conclusion, the innate immune system of the brain participates actively in neurodegenerative processes and ageing and can be triggered by peripheral insults. As a consequence behavioral changes occur.

The interaction between the cholinergic system and inflammatory mechanisms

Recently accumulating evidence suggests that there is an interplay between the cholinergic and immune system. This has been extensively investigated in the peripheral immune system. The vagal nerve is a major source of acetylcholine (Ach). It regulates visceral functions through the peripheral muscarinic ACh-receptors. Moreover, the vagal nerve has anti-inflammatory capacities by inhibiting macrophage tumour-necrosis factor (TNF) and attenuating systemic inflammatory responses\textsuperscript{54,55}. This cholinergic anti-inflammatory pathway is
mediated through the nicotinic alpha7-subunit containing receptors (α7-nAChR) on macrophages\textsuperscript{56}.

In animal models it has been shown that the α7-nAChR subunit in the brain is located on microglia and astrocytes and is involved in a cholinergic pathway which regulates microglia activation\textsuperscript{57-59}. Cholinergic stimulation down-regulates pro-inflammatory cytokine production by microglia\textsuperscript{59}. On the other hand studies have suggested that basal forebrain cholinergic neurons, which provide the major cholinergic input to the brain cortices, are vulnerable to the consequences of chronic neuroinflammation. Chronic exposure to the proinflammasome LPS produced a decline in cholinergic neurons in the basal forebrain in rats\textsuperscript{60}. Induction of inflammation by lesioning or direct injection of proinflammatory cytokines (TNF) and beta-amyloid in the basal forebrain causes impairments of cholinergic function\textsuperscript{61-63}. Collectively these studies suggest that: i) inflammatory processes may negatively influence cholinergic degeneration through toxic cytokines next to the damage due to the pathological changes in neurodegenerative diseases. ii) cholinergic neurons modulate inflammation through α7-nAChR; cholinergic deficiency could lead to a decreased inhibition of microglia activation. The subsequent heightened inflammatory actions could propagate further disease progression. It is not clear which influence these processes have on cognitive functions in humans. Decreases in α7-nAChR expression in the hippocampus and temporal cortex of AD patients have been described\textsuperscript{64}. In animal models administration of α7 agonists is beneficial to learning and memory, while antagonists produce the opposite effect\textsuperscript{65,66}. The α7nAChR is known to functionally interact with Aβ42, leading to memory dysfunction, intraneuronal accumulation of Aβ42 and tau phosphorylation\textsuperscript{62,63}. The cholinergic/inflammatory pathway is a potential target for therapeutic intervention. In \textit{in vitro} experimental studies cholinesterase inhibitors suppressed lymphocyte proliferation and the release of pro-inflammatory cytokines\textsuperscript{67,68}. Similar effects were observed when nicotine was administered\textsuperscript{69,70}. Hunter et al report in a mouse model of Down’s syndrome (a model that shares many features with AD such as plaques & tangles and neuroinflammation), that minocycline, a semi-synthetic tetracycline, inhibits microglial activation. This led to prevention of loss of cholinergic neurons in the basal forebrain and it improved cognitive performance\textsuperscript{71}. The relationship between the cholinergic system and the innate brain immune system has to be further elaborated and confirmed in human models. Full understanding of this interplay and its influence on cognition and behavior may lead to novel therapeutic approaches in neurodegenerative disease and delirium. A schematic, partly hypothetical, display of the interactions between neurodegenerative disease, the cholinergic system, inflammation and clinical symptoms is shown in Box 2.
Concluding remarks and suggestions for future research based on the thesis

Conclusions based on this thesis are summarized as follows:

1. Cholinergic deficiency probably can be clinically identified by a set of attentional tests and clinical features including apathy, hallucinations and psychomotor disturbances; this cholinergic deficiency syndrome resembles the symptoms seen in delirium.
2. The cholinergic deficiency syndrome (CDS) can serve as an indication for treatment with cholinesterase inhibitors.

3. Systemic infection causes microglia activation in the brain.

4. Ageing and neurodegenerative disease are accompanied by an inflammatory reaction in the brain; a peripheral insult can exaggerate this reaction, probably leading to clinical features resembling delirium.

5. There is evidence that cholinergic neurons and the brain immune system interact with each other; both systems are affected in neurodegenerative disease and delirium; their interplay can probably account for a part of the symptoms encountered in these diseases.

Suggestions for future research are:

- Future research should focus on the validity of the cholinergic deficiency syndrome. It is necessary to define which test are most accurate and precise to describe cholinergic deficiency profile. Independent prospective studies in larger cohorts of patients, with various neurodegenerative diseases should be performed to determine the true value of this or similar risk profiles. Recognition of this syndrome could lead to a more rational treatment of patients with cognitive dysfunction due to different nosological entities. Not only could it serve as a predictor of a satisfactory response with cholinesterase inhibitors but also caution physicians in their use of anti-cholinergic medications.

- There is considerable overlap in symptomatology between CDS and delirium. Disturbance of the cholinergic system is considered the final common pathway in delirium. Several case-reports and a single trial have reported different outcomes concerning the use of CEIs in delirium. It is worth to establish the place of these drugs in preventing or treating delirium in randomized trials. Whereas an episode of delirium may indicate cholinergic vulnerability, it may be worthwhile to study the potential value of CEIs in improving the prognosis of patients that have suffered from a delirium.
In addition to clinical symptoms, the additional value of ancillary techniques such as CSF-analysis or imaging in determining the extent of neurotransmitter deficits should be evaluated.

Increased inflammatory activity as an accompanying phenomenon in neurodegenerative disease and ageing has been well established. The concept of primed microglia, the subsequent exacerbation of immunological response in the brain after an insult and the development of behavioral deficits should be further investigated in human brains. Post-mortem studies in elderly patients should compare microglia activation in patients with and without a history of delirium. Intervention-studies could provide indirect evidence of the concept (see below).

It is not clear as to how systemic inflammation affect behavioral symptoms. Infections are accompanied by sickness behavior, comprising fever, lethargy, fatigue and anorexia. This is caused by alterations in neuroendocrine functions. An overt behavioral disturbance or delirium is probably mediated through the cholinergic/inflammatory pathway. Up till now it is not completely elucidated if a cholinergic-inflammatory pathway exists in the human brain and what its molecular constituents are. The role of the α7-nAChR subunit in the communication between cholinergic neurons and inflammatory cells has been established in animal models but requires further investigation in human models. A first step would be to identify which cells express this receptor in the brain and by which mechanisms it interacts with cholinergic neurons.

Establishment of the cholinergic/inflammatory pathway gives opportunities for different therapeutic strategies. Anti-inflammatory agents (e.g. minocycline) can reduce exaggerated inflammatory reaction which can prevent further decline of cholinergic neurons. Cholinesterase inhibitors can counteract symptomatology of cholinergic deficiency and maybe have a protective effect by reducing inflammatory reactions. Other agents such as nicotine can directly stimulate the α7-nAChR subunit to repress inflammatory activity. Although animal studies are promising, clinical trials are required to test this concept.
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