KEY TOPICS IN BRAIN RESEARCH

Edited by A. Carlsson, P. Riederer, H. Beckmann, T. Nagatsu, S. Gershon, and K. A. Jellinger

K. A. Jellinger, G. Ladurner,
and M. Windisch (eds.)

New Trends in the Diagnosis and Therapy of Alzheimer's Disease

Springer-Verlag Wien New York

Prof. Dr. K. A. Jellinger
Ludwig Boltzmann Institute of Clinical Neurobiology,
Lainz Hospital, Vienna, Austria

Prof. Dr. G. Ladurner
Department of Neurology, Landesnervenklinik,
Salzburg, Austria

Dr. M. Windisch
Department of Research and Development,
Ebewe Pharmaceuticals Ltd., Unterach, Austria

Typesetting: Best-Set Typesetter Ltd., Hong Kong
Printing: Eugen Ketterl GesmbH, A-1180 Wien
Printed on acid-free and chlorine-free bleached paper

With 33 Figures

ISSN 0934–1420
ISBN-13:978-3-211-82620-1 e-ISBN-13:978-3-7091-9376-1
DOI: 10.1007/978-3-7091-9376-1

Preface

Alzheimer's disease, the most common form of adult-onset dementia, is a major health and socio-economic problem which will continue to intensify with the increasing life expectancy and the continuing increase of the elderly in the population. Alzheimer dementia is one of the most frequent diseases in the eldery comparable in incidence to the risk of myocardial and/or cerebral infarction. It has become one of the leading causes of death in modern society. According to recent US studies the economic and social costs of Alzheimer disease patients who need substantial caregiving either by their family or public institutions were US $ 47.581 and 173.932 per case, respectively. The estimated 1991 national direct and total prevalence costs were $ 20.6 and 67.3 billion, respectively. Assuming that the prevalence of the disease remains constant, the estimated discounted present values of the direct and total costs of all current and future generations of Alzheimer's patients are $ 536 billion and $ 1.75 trillions, respectively [Ernst and Hay (1994) Am J Public Health 84: 1261]. Despite considerable progress in molecular genetic research, the causes of Alzheimer's disease are still unknown. Although the accuracy of making the clinical diagnosis of Alzheimer dementia, at least in advanced stages of the disease, is over 90%, difficulties remain in the early diagnosis and its differentation from effects of normal aging, depression and other treatable dementias. Homogeneity in clinical diagnosis and the staging of dementia using standardized criteria and modern neuroimaging methods are the basis of successful treatment strategies, the effectivity of which, up to the present, is rather limited. This volume in the series "Key Topics in Brain Research" presents the updated papers read at the 2nd International Symposium of Research Initiative EBEWE on "New Trends in the Diagnosis and Therapy of Alzheimer's Disease" held in October 1993 at Salzburg. They give an overview of our current knowledge on morphology, neuroimaging, neurochemical markers, psychopathology and drug treatment of Alzheimer disease and related dementing disorders in the elderly. Although the current possibilities of anti-dementia drug therapy are limited, and there is still lack of adequate standard treatment strategies, the use of nootropic substances targeted to specific symptoms has been proven in controlled clinical

trials. Considerable improvement of neuropsychological performance, every-day activity, and cerebral metabolism has been established. The therapeutic efficacy of these and other substances, however, must be proven in further controlled clinical trials. This volume is aimed to provide information about the current trend and limits of diagnosis and therapy of Alzheimer's disease. The Symposium and the edition of this book would not have been possible without the generous support of Ebewe Pharmaceuticals, Ltd. The secretarial help of Mr. K. Paukner, L. Boltzmann Institute of Clinical Neurobiology, Vienna, is acknowledged. Finally, we are grateful to Springer-Verlag Wien for the perfect cooperation and the excellent production of this book.

October 1994

K. Jellinger
G. Ladurner
E. Windisch

Contents

Synaptic pathology in the pathogenesis of Alzheimer dementia

R.D. Terry and **E. Masliah**

Department of Neurosciences, University of California San Diego, La Jolla, Ca, U.S.A.

Summary

Whereas the number of cortical neurons in human brain does not significantly change with age, there is a significant reduction of synaptic terminals in both normal aging brain and Alzheimer's disease (AD). Multivariant analysis of a cohort of AD patients showed a highly significant correlation between psychostatus and reduction of midfrontal synapses and large neurons, while there was no correlation with amyloid deposits. Another variable contributing to cognitive decline are numbers or neurofibrillary tangles and neuron loss in the cholinergic nucleus basalis of Meynert These and other recent morphologic data do not support the hypothesis that amyloid deposition is a major pathogenic factor of both neuronal and synaptic loss in aging and AD. The causes of synaptic pathology in AD remain to be elucidated.

Introduction

Normal, disease-free aging involves a wide variety of physiologic and structural changes in several organ systems. For example, the lungs lose elasticity and skeletal muscles undergo atrophy. Such changes in the brain are also significant, involving loss and/or shrinkage of neurons in the neocortex and elsewhere, but not everywhere. But perhaps of greater cognitive significance is the loss of neocortical synapses. In regard to neuronal populations, Brody reported in 1955 that in normal aging there is a major loss of cortical neurons, especially the smaller ones. However, he studied relatively few cases, and these were not reported as to their clinical history, nor were they examined for the presence of plaques or tangles. Subsequently, Terry et al. (1987) reported that three cortical association areas in 51 brain from clinically

normal and histologically intact individuals displayed a loss of large neurons, but an equivalent increase of small neurons, so that the total normal number of neuronal somata did not significantly change over a neurologically normal age range from 24 to 100 years. The data indicated that large neurons atrophied to join the small neuron classes.

During the past few years several reports have appeared concerning synapse populations. Scheff et al. (1993) used electron microscopy, identifying the synapse by its pre-and post-gap terminals with the characteristic post synaptic density. Our laboratory group has developed several immunochemical (Alford et al., 1993) and immunocytochemical methods (Masliah et al., 1990) which allow a larger area to be measured with greater efficiency. These later techniques depend on an immunoreaction with an antibody against synaptophysin, a protein of 38 kd which is integral to the membrane of the synaptic vesicle. The label thus localizes the presynaptic bouton. Utilizing confocal laser scanning microscopy of sections double labeled with anti-amyloid and anti-synaptophysin, we examined dorsal frontal cortex of 25 individuals (not the same people as those above) without dementia ranging from 16 to 98 years of age (Masliah et al., 1991c). An approximately linear decrease in the number of synapses per unit area was noted, and it had a correlation coefficient ® with age of -0.708, significant at 0.0001. There was a 20% difference between the group over 60 years old and those younger than 60. We propose that the loss of cortical synaptic terminals is related to shrinkage and/or loss of the projection neurons, in that a shrunken projection neuron can be presumed to be less able to maintain its normally large synaptic arbor at the distant terminal of its axon. On the other hand, local events such as altered extracellular matrix or cell membrane oat or near the synaptic terminal can not be ruled out as a cause of the synaptic loss in either normal aging or in Alzheimer disease.

As to Alzheimer disease, it must be kept in mind that the disorder is superimposed on whatever aging changes have taken place and are continuing at the time of the disease onset and throughout the course. This implies that there need be less structural or chemical difference between the normal brain and a diseased one both at advanced age than there is if the disease occurs at a younger age. Indeed, this is the case in regard to all the histologic changes. Younger victims have far more plaques, tangles, neuronal and synaptic loss than do the older ones at the same stage of dementia (Hansen et al., 1988). This would seem to be because the older ones are closer to that threshold of synaptic reserve below which dementia will become apparent. This concept also implies that if a person starts adult life with a smaller number of synapses because of genetic or, perhaps, educational or other environmental effects, the onset of the disease will be earlier.

Material and methods

As noted above, Scheff et al. (1993) have reported a highly significant loss of neocortical synapses in Alzheimer disease by the use of electron microscopy of both biopsy and autopsy tissue. We, on the other hand, have utilized three techniques on autopsy tissue: microdensitometry of peroxidase reacted anti-synaptophysin (Masliah et al., 1990), confocal quantification of fluorescent markers (Masliah et al., 1991a), and a quantitative dot-blot (Alford et al., 1993). All have shown a very severe loss (40–50%) of presynaptic terminals relative to age-matched controls. There was little or no overlap between the normals and the AD patients. When, rarely, a synapse measure was in the range of normal in an AD patient's frontal cortex, the same brain displayed a count below the normal range in the parietal lobe.

Our Alzheimer patients had undergone extensive psychometric studies during their periodic workups in the Alzheimer Disease Research Center. Included among the administered tests were the Blessed Information Memory Concentration test (IMC) (Blessed et al., 1968) as modified for American use (Fuld, 1978), the Mini-Mental State Examination (MMSE) of Folstein et al. (1975) and the Dementia Rating Scale (DRS) of Mattis (1976). The first is largely a test of memory, while the second emphasizes other cognitive abilities. The DRS has competent items which can still be answered in the later stages of the disease so that it does not reach a floor as early in the course as the other two.

Results and comments

In our series (Terry et al., 1991) as measured by the microdensitometry method, the IMC correlated with a frontal synapse score at $r = 0.729$. The correlation of the DRS with MF synapse measurement was $r = 0.673$. Step-wise regression analysis was applied in order to evaluate the relative strength of neuron counts, plaques, tangle, synapse measures and concentrations of choline acetyltransferase, all from the same three cortical areas – midfrontal (MF), superior temporal (ST), and inferior parietal (IP). These multivariate analyses also included the patients' midfrontal synapses provided .76 of the final r, inferior parietal plaques added another .15, and midfrontal plaques added .03 to the final coefficient of .94, yielding a variance of 88%. As to the MMSE the midfrontal synapses provided .73, inferior parietal plaques added .12, inferior parietal synapses another .04 and midfrontal choline acetyltransferase added 0.04 for a total coefficient of .93 and a variance of 86%. With the DRS, midfrontal synapses contributed .67, inferior parietal plaques added .25, and inferior parietal synapses .04 for a total coefficient of .96 and variance of 92% (Table 1). Those parameters not mentioned in regard to multivariant analyses did not figure significantly in the calculation. Note especially that plaques contributed only a quarter or less of the strength of these correlations. It was also interesting that the rostral superior temporal region (area 38)

Table 1. Stepwise regression: cognition/neocortex

Blessed IMC (n = 15)	
	r2
MF synapses	0.58
IP plaques	0.25
MF plaques	0.05
Final variance =	0.88 (r = .94)
MMSE (n = 15)	
	r2
MF synapses	0.53
IP plaques	0.19
IP synapses	0.08
MF ChAT	0.06
Final variance =	0.86 (r = .93)
DRS (n = 15)	
	r2
MF synapses	0.45
IP plaques	0.39
IP synapses	0.08
Final variance =	0.92 (r = .96)

did not contribute at all, while the inferior parietal samples added only a little.

In view of the apparent cognitive significance of the midfrontal synapse population, the next rational analysis was to determine which parameters might best correlate with midfrontal synapse measures. Not unexpectedly, the number of synapses in the frontal lobe correlated best with the number and health of large neurons. It was unexpected, however, that the midfrontal synaptic correlations dealt not with midfrontal neurons, but rather with those in parietal and temporal areas. Table 2 displays these relationships, showing that abnormally phosphorylated tau protein in the large layer 5 neurons of temporal and parietal areas, parietal neurofibrillary tangles and especially parietal neuron numbers are the significant correlates (Terry, Masliah, and DeTeresa, unpublished). Thus one can see that the midfrontal synaptic density, so strongly related to the loss of cognitive ability in Alzheimer disease, is itself related to the loss of those structures responsible for the

Table 2. MF synapses vs. structure in AD

	n	p	r	r2
IP Large neurons	15	0.007	+0.660	.44
IP Tangles	15	0.025	−0.576	.33
IP Layer 5 Tau-PO4	11	0.031	−0.647	.42
ST Layer 5 Tau-PO4	11	0.035–	−0.637	.41

rostral flow of associate information. The hyperphosphorylated tau immunoreaction indicates chemical and presumably functional alteration of the neuronal cytoskeleton.

The next question had to do with the role of the nucleus basalis of Meynert (nbM) with its major cholinergic connections. A single section of this nucleus at the level of the posterior border of the anterior commissure was studied in 13 cases as to numbers of neurons, plaques, tangles, and synapses (Samuel et al., 1994). Indeed, this region turned out to be of great importance in regard to memory tests, especially in the Blessed IMC. This test was best predicted as to variance (r2) by nbM tangles — 53%, and MF synapses — 26%, plus nbM neuron count 7%, for a total variance of 86% (r = .93). nbM tangles, when added to the parameters from the neocortical regions added 24% in the MMSE to 50% from frontal synapses, 13% bnM neurons, 8% nbM synapses, and 5% frontal plaques, totaling the variance at 100%. The addition of the nbM parameters also changes the step-wise regression of the Dementia Rating Scale. In step 1, nbM tangles providing 51%, step 2 included MF synapses up to 73% finally, MF plaques provided an additional 9%, so that the total variance for the DRS was 82% (Table 3). The conceptualization component of the DRS correlated strongly with the density of frontal synapses.

Until these studies were performed, we had considered the MMSE and the IMC to reveal very similar cognitive abilities, but we now see how they are functionally different and relate to different areas of the brain. Cognition relates most closely to the frontal lobe, while memory is more strongly related to the cholinergic nbM:

Table 3. Stepwise regressions: cognition/neocortex and nbM

Blessed IMC (n = 13)	
	r2
nbM tangles	0.53
MF synapses	0.26
nbM neurons	0.07
Final variance =	0.86 (r = .93)
MMSE (n = 13)	
	r2
MF synapses	0.50
nbM tangles	0.24
nbM neurons	0.13
Final variance =	0.87 (r = .93)
DRS (n = 13)	
	r2
nbM tangles	0.51
MF synapses	0.22
MF plaques	0.09
Final variance =	0.82 (r = .91)

Plasticity is quite active in the Alzheimer neocortex as evidenced by the presence of abnormal sprouts and many processes containing GAP43 (Masliah et al., 1991b) or tau (Ihara, 1988). Amyloid precursor protein is often colocalized within these sprouts (Masliah et al., 1992). But if there are new terminals formed in the neocortex during the disease, their number does not at all keep with the loss of synapses (Catman et al., 1992). The limbic region, on the other hand, being a much more primitive part of the brain, has far greater plastic capacity. Except in layer two of the entorhinal cortex, the synapse number in that cortex is maintained at a normal level in the disease (Masliah et al., 1991c). This is probably partly the result of reactive sprouting and undoubtedly involves erroneous transmitter system. The neuron number in these deeper layers is relatively preserved. Similarly, the innermost third of the hippocampal molecular layer displays an actual increase in synapse number (Hyman et al., 1984; Masliah et al., 1991c; Strubel and Clark, 1992). Again, this is the result of regenerative sprouting, and again with an erroneous neurotransmitter; that is, cholinergic rather than glutaminergic. Given the devastating loss of neurons in entorhinal layer two, which is the source of the perforant path (Hyman et al., 1984), it is not surprising that the outer twothirds of the hippocampal molecular layer displayed decreased synaptic density. Plasticity in the olfactory bulb (Strubel and Clark, 1992) and in the hippocampal region probably provides erroneous transmitters and might well be detrimental to normal function rather than restorative.

The Alzheimer research literature is heavily laden with studies on amyloid, and yet in the data recited above, plaque numbers contribute relatively little to the strength of the functional correlations. Several recent reports bear out the failure of plaque numbers, or plaque burden (Verano et al., 1990; Hyman et al., 1993) to correlate with the severity of Alzheimer dementia. Neocortical plaque volume amounts to between 5 and 10% of the neocortex, and that is about the strength of most plaque correlations. While amyloid is undoubtedly toxic to neurons in vitro (Yankner et al., 1990), this effect has not been adequately demonstrated in vivo (Podlisny et al., 1993). Most synaptic loss in the cortex is in the neuropil between plaques where there is no amyloid. Furthermore, there is no gradient of neuropil damage extending outward from the plaque. In the AD retina, ganglion cells are lost in the absence of both amyloid and tangles (Blanks et al., 1989).

Given the relatively minor role of amyloid in terms of its correlation with dementia, it should not be expected that arresting the deposition of that protein, however prominent, would do very much toward reduction of the severity of the disease or slowing its course. Greater emphasis must be given to maintenance of normal synaptic distribution with normal transmitter activity. The normal synaptic activity must be maintained for normal cognition. Trophic factors and membrane maintenance factors might well be considered as potential therapeutic

agents, especially if they could be administered early in the course of the disease. The recent very important findings concerning apolipoprotein E and especially the E4 variety (Corder et al., 1993) may well open new avenues in those directions, in that the protein is a cholesterol carrier, and a malfunction in this area could well destabilize neuronal membranes.

References

Alford MF, Masliah E, Hansen LA, Terry RD (1993) A simple dot-immunobinding assay for the quantification of synaptophysin-like immunoreactivity in the human brain. J Histochem Cytochem 42:283–287

Blanks JC, Hinton DR, Sandun AA, Miller CA (1989) Retinal ganglion cell degeneration in Alzheimer's disease. Brain Res 501:364–372

Blessed G, Tomlinson BE, Roth M (1988) The association between quantitative measures of dementia and of senile changes in the cerebral grey matter of elderly subjects. Br J Psychiatry 114:797–811

Brody H (1955) Organization of the cerebral cortex. III. A study of aging in the human cerebral cortex. J Comp Neurol 102:511–556

Corder EH, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Peicak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921–923

Cotman CW, Cummings BJ, Whitson (1991) The role of misdirect plasticity in plaque biogenesis and Alzheimer's disease pathology. In: Hefti F, Brachet P, Will B, Christen Y (eds) Growth factors and Alzheimer's disease. Springer, New York, pp 222–233

Folstein MF, Folstein SE, McHugh PR (1975) "Mini-mental state": a practical method for grading the cognitive state of patients for the clinician. J Psychiatry Res 12:189–198

Fuld PA (1978) Psychological testing in the differential diagnosis of the dementia. In: Katzman R, Terry RD, Bick KL (eds) Alzheimer's disease: senile dementia and related diseases. Raven Press, New York, pp 185–196

Hamos JE, DeGennaro LJ, Drachman DA (1989) Synaptic loss in Alzheimer's disease and other dementias. Neurology 39:344–361

Hansen LA, DeTeresa R, Davies P, Terry R (1988) Neocortical morphometry, lesion counts, and choline acetyltransferase levels in the age spectrum of Alzheimer's disease. Neurology 38:48–54

Hyman BT, Marzloff K, Arriagada PV (1993) Lack of accumulation of senile plaques or amyloid burden in aging, Alzheimer's disease and Down syndrome (Abstract). J Neuropathol Exp Neurol 52:264

Hyman BT, Van Hoesen GW, Damasio AR, Barnes CL (1984) Alzheimer's disease: cell-specific pathology isolates the hippocampal formation. Science 225:1168–1170

Ihara Y (1988) Massive somatodendritic sprouting of cortical neurons in Alzheimer's disease. Brain Res 459:138–144

Masliah E, Fagan AM, Terry RD, DeTeresa R, Mallory M, Gage FH (1991a) Reactive synaptogenesis assessed by synaptophysin immunoreactivity is

associated with GAP-43 in the dentate gyrus of the adult rat. Exp Neurol 113:131–142

Masliah E, Mallory M, Hansen L, Alford M, Albright T, DeTeresa R, Terry RD, Baudier J, Saitoh T (1991b) Patterns of aberrant sprouting in Alzheimer disease. Neuron 6:729–739

Masliah E, Mallory M, Hansen L, Alford M, DeTeresa R, Terry R, Baudier J, Saitoh T (1992) Localization of amyloid precursor protein in GAP43-immunoreactive aberrant sprouting neurites in Alzheimer's disease. Brain Res 574:312–316

Masliah E, Terry RD, Alford M, DeTeresa R (1990) Quantitative immunohistochemistry of synaptophysin in human neocortex: an alternative method to estimate density of presynaptic terminals in paraffin sections. J Histochem Cytochem 38:837–844

Masliah E, Terry RD, Alford M, DeTeresa RM, Hansen LA (1991c) Cortical and subcortical patterns of synaptophysin-like immunoreactivity in Alzheimer disease. Am J Pathol 183:235–246

Mattis S (1991) Mental status examination for organic mental syndrome in the elderly patients. In: Bellack L, Karasu TB (eds) Geriatric psychiatry. Grune and Stratton, New York, pp 77–121

Podlisny MB, Stephenson DT, Frosch MP, Tolan DR, Lieberburg I, Clemens JA, Selkoe DJ (1993) Microinjection of synthetic amyloid β-protein in monkey cerebral cortex fails to produce acute neurotoxicity. Am J Pathol 142:17–24

Samuel W, Terry RD, DeTeresa R, Butters N, Masliah E (1994) Clinical correlates of cortical and nucleus basalis pathology in Alzheimer dementia. Arch Neurol 51:772-778

Scheff SW, Price DA (1993) Synapse loss in the temporal lobe in Alzheimer's disease. Ann Neurol 33:190–199

Strubel RG, Clark HB (1992) Olfactory bulb lesions in Alzheimer's disease. Neurobiol Aging 13:469–473

Terry RD, DeTeresa R, Hansen LA (1987) Neocortical cell counts in normal human adult aging. Ann Neurol 21:530–539

Terry RD, Masliah E, Salmon DP, Butters N, DeTeresa R, Hill R, Hansen LA, Katzman R (1991) Physical basis of cognitive alterations in Alzheimer disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572–580

Verano A, Chandran R, Faraldi F, Bennet D, Reyes MG (1990) Volume density of senile plaques in Alzheimer's disease (Abstract). J Neuropathol Exp Neurol 52:264

Yankner BA, Duffy LK, Kirschner DA (1990) Neurotrophic and neurotoxic effects of amyloid β protein: reversal by tachykinin neuropeptides. Science 250:279–282

Correspondence: R.D. Terry, M.D., Department of Neurosciences, University of California San Diego, 9500 Gilman Drive, La Jolla, Ca 92093-0624, U.S.A.

Classification of dementias based on functional morphology

K.A. Jellinger and **C. Bancher**

Ludwig Boltzmann Institute of Clinical Neurobiology, Lainz Hospital, Vienna, Austria

Summary

Dementia syndromes in adults can be caused by many different conditions that find their pathological correlates in seven major groups of CNS disorders: 1. presenile and senile dementia of the Alzheimer type (AD/DAT), the diagnosis of which is based on quantitative assessment of neuritic changes — neuritic plaques, neurofibrillary tangles — probably representing end-stage markers or epiphenomena of primary neuronal degeneration of hitherto unknown origin; 2. other degenerative diseases, e.g. Parkinson's disease associated with AD, Diffuse Lewy body disease, Pick's lobar atrophy, Huntington's disease, progressive supranuclear palsy, corticobasal degeneration, and multiple system atrophies; 3. vascular dementia (VD) with several subtypes — multiinfarct encephalopathy, strategic and small vessel infarct dementia (multilacunar state, subcortical type Binswanger, granular cortical atrophy); 4. mixed type dementia (MIX) due to coexistence of AD/DAT and vascular pathologies; 5. Prion diseases or transmissible spongiform encephalopathies (e.g. Creutzfeldt-Jakob disease); 6. normal pressure hydrocephalus; 7. various organic brain diseases, such as tumors, chronic infections, alcoholic and metabolic encephalopathies. In large autopsy series, AD/DAT account for 70 to 80%, VD and MIX for 8 to 10%, each while the remaining disorders are of minor importance. While most cohorts show VD as the second most frequent type of dementia, in some series it is Lewy body dementia. In a personal consecutive postmortem series of 1200 aged subjects (mean age 79.5% years), 78% fulfilled the pathologic criteria for AD, but only 43% were "pure" forms, 15.5% had additional minor vascular lesions, 10% Lewy body pathology, 5.5% were MIX type dementia, 13% VD with no or only little AD pathology; 8.7% revealed other CNS disorders, and 0.6% displayed no abnormality beyond age-related changes. While the pathogenesis of rare dementia forms, e.g. infections, is well elucidated, for the majority of degenerative dementia disorders including AD/DAT, the etiology and pathogenesis are poorly understood. The morphologic diagnosis and clinico-pathological correlations of degenerative dementias need more stand-

---

* Dedicated to Prof. Dr. H. Orthner, Göttingen, on the occasion of his 80th anniversary

ardized criteria; elucidation of their etiology as a basis of treatment strategies is subject of intense current research efforts of basic and clinical neurosciences.

Introduction

Dementia, a syndrome of acquired global disturbances of higher cerebral functions and cognition without change of alertnes represents one of the major health problems facing modern society. With the increasing life expectancy, the prevalence of dementias has risen considerably, from 1% in those aged 60 to 65 years to over 40% in the ninth decade (Hofman, 1993; Katzman and Kawan, 1994). Dementia can be caused by many different conditions, some of which are treatable or reversible (Arnold and Kumar, 1993; Wolters and Scheltens, 1993). The most widely used clinical classifications, the DSM-III-R (1987) and WHO ICD-9 and 10 (Lauter et al., 1990; Förstl et al., 1993) distinguish several groups of progressive dementia syndromes in adults, i.e. presenile, senile and other organic disorders that correlate with several major morphological groups of central nervous system (CNS) diseases. In most clinical and autopsy series, degenerative dementia of the Alzheimer type (DAT) is the most frequent cause of mental decline in the elderly; it accounts for 65 to 80% (Jellinger et al., 1990). Vascular dementia (VD) caused by cerebrovascular lesions, and mixed type dementia (MIX) due to coexistence of both AD and vascular pathologies represent 8 to 10% each, with a wide range for VD between 3 and 85% (Jellinger et al., 1990; Roman et al., 1993). Other neurodegenerative disorders including Parkinson's, Pick's, Huntington's, or Diffuse Lewy body disease (DLBD), multiple system atrophies (MSA), etc., account for 7 to 10%, other nondegenerative organic CNS disorders for 3 to 5%, while in less than 1% no pathologic CNS changes are found (Fig. 1). The post mortem diagnoses in a personal consecutive autopsy

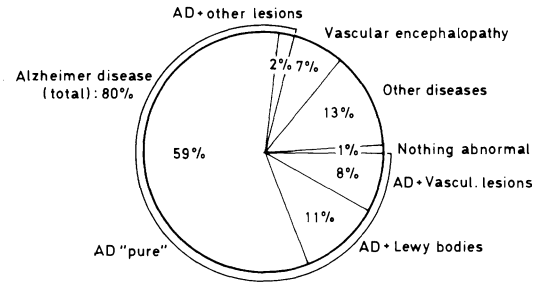

Fig. 1. Neuropathological findings in elderly demented subjects; 1845 cases from recent literature (Alafuzoff et al., 1987; Joachim et al., 1988; Boller et al., 1989; Sulkova et al., 1989; Jellinger et al., 1990; Mirra et al., 1991; Gilleard et al., 1992; Mendez et al., 1992; Galasko et al., 1993; Jodregui et al., 1993)

Table 1. Causes of dementia in autopsy series of aged individuals (1984–1993)

555 M/645 F	n	%	Age (mean ± SD)
"Pure" AD/DAT	516	43.0	82.1 ± 4.6
DAT + hemorrhage	28	2.3	79.8 ± 7.2
DAT + vascular lesions (CVD)	185	15.5	82.6 ± 5.5
DAT + Parkinson's disease	105	8.7	78.0 ± 7.1
DAT, Lewy body variant/DAT + DLBD	13	1.1	77.3 ± 4.5
DAT + incidental Lewy bodies	4	0.3	80.0 ± 4.0
DAT + CVD/MIX dementia	66	5.5	83.2 ± 5.5
DAT + tumor, other lesions*	16	1.4	75.7 ± 4.7
DAT total	933	77.8	80.3 ± 5.7
Vascular encephalopathy (VE)[1]	155	12.9	78.2 ± 8.3
Other syndromes	105	8.7	74.0 ± 8.3
Nothing abnormal	7	0.6	85.7 ± 3.6
TOTAL	1200	100.0	79.5 ± 6.7

* DAT + tumors (6); DAT + encephalitis (4), DAT + Wernicke's encephalopathy (4), AD + ALS (2)

[1] JCD (22), Huntington d. (11), Pick's d. (10), Wernicke d. (9), PD (9), PSP (7), MS (6), Frontotemp. deg. (5), MSA (5), thalamic/hippocampal sclerosis (4), CNS lymphoma (3), posttraumat. encephalop. (3), DLBD (2), OLD (2), CBD (2), PD + ALS (1), glial dystrophy (1), Fahr's disease (1)

series of a large general and geriatric hospital (1984–1993) are given in Table 1. For comparison, the causes of dementia in a recent cohort of 50 aged subjects seen among 200 consecutive neuropathological autopsies are given in Table 2. For some of these conditions, the causes and pathogenesis of cerebral dysfunction leading to dementia are well understood, e.g. CNS infections, toxic of metabolic disorders. The most frequent dementia syndromes, however, belong to the large group of neurodegenerative diseases, the etiology of which are still enigmatic. The present overview attempts to classify the most important substrates of dementia on a neuropathological basis and to present some recent data on their etiology and pathogenesis.

Alzheimer disease and senile dementia of Alzheimer type (AD/DAT)

This neurodegenerative disease is the most common form of adult-onset dementia. A community-based study suggested that about four million persons in the United States have AD/DAT, and their number may rise to 14 millions by the year 2040 (Bachmann et al., 1993). Even though the accuracy of clinical diagnosis of AD using established

Table 2. Causes of dementia in autopsy series of aged individuals; January 1–June 30, 1994 (among 200 autopsy cases)

12 males/38 females	n	%	Age (mean ± SD)
"Pure" AD/DAT	16	32	78.0 ± 4.5
DAT + CVD (vascul. lesions)	10	20	86.8 ± 4.9
DAT + Parkinson's disease	5	10	86.2 ± 2.1
DAT, Lewy body variant/DAT + DLBD	4	8	83.2 ± 3.8
DAT + incidental Lewy bodies	2	4	83.0
DAt + VE/MIX dementia	3	6	88.3 ± 4.2
DAT total	40	80	82.5 ± 4.3
Vascular encephalopathy (VE)	4	8	81.3 ± 1.1
Creutzfeldt-Jakob disease	3	6	71.4 ± 2.3
Diffuse Lewy body disease	1	2	78.0
PD + int. hydrocephalus	1	2	74.0
Nothing abnormal	1	2	83.0
Non-Alzheimer diseases	10	20	77.2 ± 2.6
TOTAL	50		81.4 ± 3.8

criteria (McKhann et al., 1984) is up to 90–95% in recent selected series (Jellinger et al., 1990), the definite diagnosis of AD can only be established by histopathologic examination of the brain (Khachaturian, 1985; Tierney et al., 1985; Mirra et al., 1993). The morphology of AD/DAT is featured by a number of changes – brain atrophy, loss of neurons and synapses, granulovacuolar degeneration, gliosis, senile plaques (SP), neurofibrillary tangles (NFT), neuropil threads (NPT), and amyloid angiopathy – among which only the classical AD markers – SP and NFT – are of diagnostic significance. The current morphologic diagnosis of AD/DAT is based on the (semi)quantitative assessment of SP and NFT (Table 3). Since both lesions also occur in brains of non-demented aged individuals (rev. Jellinger, 1993; Crystal et al., 1993), many neuropathologists consider the post mortem diagnosis of AD a problem especially when reliable clinical information is lacking (Bancher et al., 1993). There is no agreement on whether SP or NFT should be attributed greater diagnostic value; major difficulties arise in cases with only moderate AD pathology, since they may represent the "gray zone" between normal aging, incipient (subclinical) and fullfledged AD/DAT (Morris et al., 1991; Braak and Braak, 1991; Dickson et al., 1991; Arriagada et al., 1992; Price et al., 1992; McKee et al., 1992; Berg et al., 1993).

Senile plaques (SP) are complex lesions composed of focal extracellular deposits of amyloid βA4 protein (Aβ) later surrounded by dystrophic neurites and glial elements (see Jellinger, 1993; Mirra et al.,

Table 3. Histological criteria of Alzheimer disease

1. Criteria according to Khachaturian (1985)
   Senile (SP) or neuritic plaques (NP) in the presence of
   neurofibrillary degeneration (NFT) in neocortex (any region)
   a) age < 50 a: 2–5/mm² c) age 66–75 a: >10/mm²
   b) age 50–60 a: >=8/mm² d) age > 75 a: >15/mm²
2. Criteria according to Tierney et al. (1988) Lesion/x25 field
   A 1: one/several SP + NFT in hippocampus (without neocortex)
   A 2: one/several SP + NFT in neocortex + hippocampus
   A 3: one/several SP + NFT in neocortex (without hippocampus)
3. CERAD criteria (Mirra et al., 1993)
   a) definite AD: "C" age-adapted plaque score*
   clinical dementia
   other dementive lesions absent
   b) probable AD: "B" age-adapted plaque score
   clinical dementia
   presence or absence of other lesions
   causing or related to dementia

*Age-adapted plaque score

Age at death	Frequence of plaques			
	none	few	moderate	numerous
<50 a	0	C	C	C
50–75	0	B	C	C
>75	0	A	B	C

A uncertain; *B* probable; *C* definite

1993; Benzing et al., 1993a, b; Masliah et al., 1993). Aβ is a proteolytic fragment of the β amyloid precursor protein (βAPP) that comprises a complex group of membrane-anchored polypeptides that undergo proteolytic cleavage (Selkoe, 1993). Two basic mechanisms proposed to explain the formation of SP are: a) that plaque formation initiates with Aβ deposition and the toxic effects of this molecule induce neuritic degeneration (Hyman and Tanzi, 1992; Selkoe, 1993; Mulhaupt et al., 1993; Mattson et al., 1993; Mullan and Crawford, 1993); b) that plaque formation results from the combined effect of synaptic and axonal injury followed by βA deposition and cycles of neuritic sprouting and degeneration (Pappola et al., 1991; Masliah et al., 1992, 1993; Sparks et al., 1993). It has been hypothesized that abnormal processing of βAPP related to genetic abnormalities and age-related changes in brain metabolism is central to the AD process causing a cascade of pathologic events that culminate in the formation of SP, neuritic changes and cell death ("amyloid cascade theory" – Hyman and Tanzi, 1992; Selkoe, 1994; Mattson et al., 1993; Delacourte, 1993). However, since many amorphous ("diffuse") Aβ deposits in brain tissue are virtually devoid of detectable alteration of neurons or synapes (Benzing et al., 1993; Masliah et al., 1992) and show a spatial pattern independent from that

of NFT and NPT (Braak and Braak, 1991, 1994; Price et al., 1992; Lassmann et al., 1992; Arriagada et al., 1992; Armstrong et al., 1993; Delaere et al., 1993), the mere presence of Aβ in brain tissue seems insufficient to induce AD cytopathology and to produce dementia. This concern is supported by the fact that Aβ deposits are almost constant findings in brains of nondemented very old subjects (Braak and Braak, 1991, 1994; Morris et al., 1991; Delaere et al., 1991, 1993; Dickson et al., 1991; Price et al., 1992; Arriagada et al., 1992; Sparks et al., 1993; Berg et al., 1993; Crystal et al., 1993) and that their amount in the brain tissue – although increasing with age (Price et al., 1992; Dickson et al., 1991; Berg et al., 1993; Delaere et al., 1993; Coria et al., 1993; MacKenzie, 1994) – does not show any correlation with the severity and progression of the disease (Braak and Braak, 1991; Dickson et al., 1991; Lassmann et al., 1992; Price et al., 1991; Delaere et al., 1991, 1993; DeKosky et al., 1992; Armstrong et al., 1993; Kazee et al., 1993; Crystal et al., 1993; Bennet et al., 1993).

On the other hand, studies of the molecular composition of the abnormal neuritic processes of SP have shown that, at least in early stages, they retain their transmitter-phenotype (Benzing et al., 1993a, b) and are immunoreactive with antibodies against synaptic axonal and/or cytoskeletal proteins (Pappola et al., 1991; Arai et al., 1992; Masliah et al., 1992, 1993; Sparks et al., 1993). These data support the hypothesis of primary synaptic/neuronal and/or axonal damage in SP formation (Pappola et al., 1991; Sparks et al., 1993; Masliah et al., 1994).

Neurofibrillary tangles (NFT) are neuronal cytoplasmic collections composed of bundles of paired helical filaments (PHF), highly insoluble cross-linked protein polymers containing hyperphosphorylated tau proteins, resulting from abnormal phosphorylation of this microtubule-associated protein followed by progressive ubiquitination caused by defects in neuronal cytoskeletal protein processing (Mena et al., 1991; Bancher et al., 1991; Delacourte, 1993; Goedert, 1993; Braak et al., 1994). PHF are also diffusely distributed within dendritic processes of the neuropil as NPT (Masliah et al., 1993; Markesberry et al., 1993) and in neuritic plaques, in the formation of which the temporal sequence of Aβ deposition and dystrophic neuritic changes is still a matter of discussion (Pappola et al., 1991; Benzing et al., 1993a, b; Sparks et al., 1993; Masliah et al., 1993).

Despite considerable progress in the characterization of AD lesions the role of both SP and NFT in the pathogenesis of cerebral dysfunction is poorly understood. Although the intensity of neuritic DAT pathology usually shows much better correlation than that of Aβ deposits with both mental impairment (Arriagade et al., 1993; Crystal et al., 1993; Delaere et al., 1991; Lassmann et al., 1992; McKee et al., 1992; Mena et al., 1991; Morris et al., 1991) and biochemical changes, such as cortical choline acetyltransferase (ChAT) activity and synaptic markers (Esiri et al., 1990; Terry et al., 1991; Lassmann et al., 1992; DeKosky et al., 1992;

Lehericy et al., 1993), the presence of neuritic DAT markers is not necessarily associated with mental decline. In nondemented aged persons with large numbers of Aβ diffuse SP in neocortex, in general, fewer numbers of NFT and neuritic SP occur in hippocampus in mildly demented subjects, whereas in more advanced dementia, the major difference is an increase in the number of neocortical NFT and shift from "primitive" to neuritic SP (Arriagade et al., 1993; Kazee et al., 1993; Price et al., 1991). Although NP and NFT in neocortex are a constant morphological feature associated with dementia (Crystal et al., 1993; McKee et al., 1992; Mena et al., 1993), several prospective clinicopathological studies revealed relatively poor correlations between neuritic lesion counts in both isocortex and hippocampus and the severity of dementia, particularly in mildly affected individuals (Dickson et al., 1991; Jellinger et al., 1991; Delaere et al., 1991, 1993; Terry et al., 1991; Morris et al., 1991; Lassmann et al., 1992; Price et al., 1992; McKee et al., 1992; Arriagada et al., 1992; Berg et al., 1993).

Hence, neuritic lesion densities in both areas may not differentiate between cognitively normal aged controls and DAT patients (McKeel et al., 1993). Others have related the severity and duration of dementia to the accumulation of NFTs in the nucleus basalis of Meynert (Samuel et al., 1994). In general, the younger the patient at the onset of dementia and at the time of death, the greater is the burden of NP and NFT, while patients with DAT do not have an inevitable age-dependent increase of NP and NFT pathology (Kazee et al., 1993; Mena et al., 1993; Bennett et al., 1993). Neuritic DAT markers even may decrease in frequency with age (Crystal et al., 1993; Mann, 1994). These data suggest that neuritic lesions may represent end-stage markers of a neurodegenerative process probably related to a generalized cytoskeletal disorder in AD causing mental impairment and that NFT pathology does not develop as a mere consequence of the neurotoxic action of Aβ (Goedert, 1993), while the progression of dementia in DAT patients is associated with specific patterns of neuropathological change.

Another morphological variable that shows better correlation with the severity of dementia than the mere number of NFT and SP in the cortex is a hierarchical topographic spreading of NFT pathology from the inferomedial temporal allocortex over large areas of the neocortex which is highly independent of the pattern and quantity of Aβ deposits (Dickson et al., 1991; Van Hoesen et al., 1991; McKee et al., 1992; Jellinger et al., 1991; Berg et al., 1993; Braak and Braak, 1992, 1994). The neuritic AD process appears to involve early the pyramidal neurons of the superficial pre-α II layer of the transentorhinal and entorhinal region in the anterior parahippocampal cortex, possibly due to enhancement of their high content of βAPP during age-related resprouting (Roberts et al., 1993). These neurons are labelled by the antibody AT8 recognizing two Ser Pro motifs of abnormally phosphorylated tau in a pretangle stage in adults (Braak et al., 1994). The pyramidal cells of the

superficial entorhinal region are the origin of the glutamatergic "perforant" pathway, a major relay and control gate between both the hippocampus and the amygdala and many isocortical association areas and limbic circuits (Hyman et al., 1990; Van Hoesen et al., 1991; Braak and Braak, 1992, 1994; Jones, 1993). Damage to the entorhinal region affected by AD neuritic pathology will cause severe synaptic loss in hippocampus due to its disconnection from isocortical inputs (Van Hoesen et al., 1991; Honer et al., 1992). It thus may contribute to early memory or cognitive deficits in both aging and AD/DAT (Hyman et al., 1990; Braak and Braak, 1992; McKee et al., 1992; Price et al., 1992; Bancher et al., 1993). The initial "transentorhinal" stages are followed by affection of the total entorhinal region with spreading of neuritic AD changes to the hippocampus but preservation of the isocortex. Since some of the nondemented old subjects show neuritic AD changes restricted to the allocortex (Dickson et al., 1991; Price et al., 1992; McKee et al., 1992; Berg et al., 1993; Jellinger, 1993; Braak et al., 1993; Arriagada et al., 1992, 1993; McKeel et al., 1993; Bouras et al., 1993), it has been suggested that this "limbic" stage may represent a morphologic correlate of initial stages of AD (Bancher et al., 1993; Hubbard et al., 1990). In the final "isocortical" stages severe affection of both the hippocampus and many isocortical association areas by the destructive process related to neuritic AD pathology causes disruption of intracortical, cortico-cortical and subcortical connections (Markesberry et al., 1993; De Lacoste et al., 1993; Braak and Braak, 1994). The resulting global cortico-cortical disconnection syndrome appears to be a major correlate of dementia in AD/DAT (Table 4).

Comparative studies of the neuropathologic staging of neuritic AD pathology (Braak and Braak, 1991) with psychometrically assessed mental status using Blessed test scores (Braak et al., 1993) or mini-mental state (Bancher et al., 1993) in prospective cohorts of aged individuals showed a significant linear correlation between both param-

Table 4. Clinico-pathological correlates in Alzheimer's disease

1. *Allocortex:* Deafferentation of hippocampus due to initial involvement of the (trans)entorhinal region by neuritic AD lesions ⊃ early cognitive, olfactory and mnestic deficits
2. *Isocortex:* hierarchical involvement of isocortical association areas by neuritic AD lesions:
   a) temporal isocortex: degeneration of cortico-cortical connections to prefrontal and parietal areas ⊃ visuo-spatial, speech, auditive disorders;
   b) occipital cortex: rare visual variant of AD (Balint syndrome)
   Progressive degeneration of intracortical, cortico-cortical connections ⊃ global cortico-cortical disconnection ⊃ dementia
3. *Subcortex:* degeneration of cortico-subcortical neuronal systems (neuronal loss in subcortical projection nuclei) ⊃ primary sensory deafferentation ⊃ affective, extrapyramidal, vegetative disorders

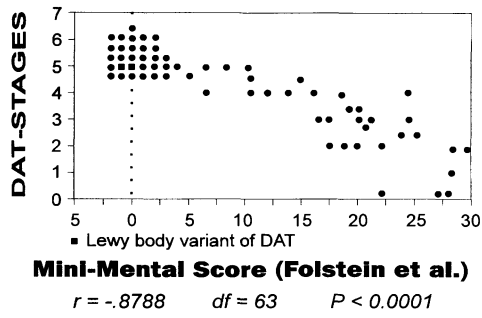

Fig. 2. Correlation between Mini-Mental scores and neuritic Alzheimer stages in 63 consecutive autopsies of aged individuals (mean age 79.9 yrs)

eters (Fig. 2). These data suggest that the hierarchical topographic spreading of neuritic AD changes may represent a better reflection of the progression of degeneration in AD than purely numerical lesion counts and may give information about the natural history of the dementing disease process.

In order to improve the evaluation criteria of AD we compared the currently used diagnostic criteria and the staging of neuritic AD changes with graded psychostatus (MMS, performed not longer than 4–6 months prior to death) in a consecutive autopsy series of 60 elderly (14 males, 46 females aged 54 to 97, mean 79.6 years) from the Vienna Prospective Longitudinal Study of Dementia (Jellinger et al., 1991). Neuropathologic assessment was performed independently by two neuropathologists (K.J., C.B.) on paraffin-embedded sections using modified Bielschowsky and Reusche's silver stains. The data summarized in Table 5 show excellent correlations between all three morphologic AD criteria and neuritic AD staging in severely demented patients (MMS 10–0), all with clinical diagnosis of severe or probable AD. Only two brains with neuritic AD lesions almost restricted to the allocortex (Tierney et al. A-1) showed negative CERAD criteria. Among 17 moderately demented subjects (MMS 11–23) with clinically probable or possible AD, six of them were Khachaturian negative, 13 were CERAD negative; eight of them scored Tierney negative. In 12 of these brains neuritic AD lesions were restricted to the limbic regions (stages 2–4); four of them (subjects aged 91 to 99 years with MMS 12–20) revealed only neurofibrillary tangels (NFT) in the allocortex with no or only very few amyloid plaques in two cases each. Among nine age-matched controls (mean 78.5 years) with no or only mild cognitive changes (MMS 24–30), five were Khachaturian positive, but all were CERAD negative; two scored Tierney et al. A 1, with neuritic AD changes limited to the allocortex, i.e. scoring Braak & Braak stages 1 to 4, while two brains showed multiple amyloid plaques without neuritic AD

Table 5. Relationship between psychostatus (MMS) and morphological Alzheimer disease criteria in consecutive autopsy series of 60 elderly subjects

MMS		0–10 (n = 34)	11–23 (n = 17)	24–30 (n = 9)
Mean age (yrs)		80.4	85.9	78.5
Morphology criteria				
Khachaturian	+	34	11	5
Tierney et al.	A2	32	3	0
	A1	2	6	2
	neg.	0	8	7
CERAD-	C (definite AD)	30	0	0
	B (probable AD)	2	4	0
	A (uncertain)	1	3	0
	negative	1	10	9
Braak & Braak	stage 6	13	0	0
	stage 5	18	0	0
	stage 4–5	2	3	0
	stage 4	1	4	1
	stage 3	0	5	1
	stage 2	0	3	4
	stage 1	0	0	1
	stage 0	0	1	2

changes. Statistical evaluation of the relationship between MMS and the different morphologic AD criteria by non-parametric Spearman correlation analysis revealed a highly significant correlation for both CERAD (r −.8755) and Braak & Braak (−.8788), but much less for Tierney et al. (r −.8238) and worst for Khachaturian (r −.4558). Multivariate analysis showed highly significant correlations between MMS and both CERAD (sig T 0.0000) and Braak & Braak (0.0003), much less for Tierney et al. (sig T 0.02307) and rather bad correlation for Khachaturian criteria (sig T 0.7993). These data confirm the excellent agreement between graded psychostatus and both Braak & Braak staging of neuritic AD changes and the CERAD criteria, the latter showing, at least in our material, slighly higher significant correlation than the Braak & Braak staging. There is less concordance with the Tierney et al. criteria, while the NIA criteria (Khachaturian, 1985) may be falsely positive in cognitively intact individuals not fitting into the other diagnostic AD criteria. However, there are a few brains of mildly demented or cognitively normal elderly subjects with either large numbers of diffuse plaques or NFTs mainly in the allocortex with no or very few amyloid deposits (Ulrich et al., 1992; Bancher and Jellinger, 1994) that show high variability in morphologic interpretation or may escape the currently used diagnostic criteria of AD. (Mirra et al., 1994). These and other "borderline" cases appear worthy of specific documentation in order to reduce disagreement in neuropathological diagnosis and clinico-pathologic evaluation.

Another parameter that parallels well with cognitive dysfunction is a reduction of synapses and small vesicle synaptic proteins in frontal,

and temporal neocortex, and hippocampus (DeKosky et al., 1990; Terry et al., 1994; Lassmann et al., 1992; Honer et al., 1992; Masliah et al., 1993; Scheff and Price, 1993; Terry et al., 1994). Another important microscopic feature of both aging brain and AD/DAT is a considerable loss and shrinkage of large neurons in the neocortex particularly in temporal and parietal lobes, in hippocampus and in the cholinergic nucleus basalis of Meynert (see Masliah et al., 1993; Samuel et al., 1994; Terry et al., 1994). This may, at least in part, be related to increased cell death in AD brain as recently demonstrated by DNA fragmentation in situ (Lassmann et al., 1994). Although there is a good correlation between the loss of synapses or their markers and neuritic AD changes (Terry et al., 1991; Lassmann et al., 1992), the causal relationship between these two types of lesions is unclear since there is no evidence for a spatial relation between synapse density or intensitiy of their biochemical markers in the neuropil and neuritic AD pathology (Terry et al., 1991; Masliah et al., 1992; DeKosky et al., 1992; Lassmann et al., 1992; Bennett et al., 1993). However, synaptic loss is suggested to reflect neuronal degeneration in specific areas of the allo- and isocortex affected by a hitherto unknown pathologic process that ultimately leads to neuronal death and disruption of intracortical, cortico-cortical and cortico-subcortical connections as a/the major cause of dementia (Hof and Morrison, 1994), while the so-called morphological AD markers – SP and NFT – may represent a non-specific response of the neuronal network to the underlying process the molecular basis and pathogenesis of which await further elucidation.

Other dementing neurodegenerative diseases

Neurodegenerative diseases frequently associated with mental dysfunction are summarized in Table 6. Most of these disorders, in addition to systemic neuronal loss and gliosis, are characterized by intracellular neuronal and/or glial inclusions made of components of the cytoskeleton that, for as yet unknown causes, escape proteolytic degradation. Ultrastructurally, the NFT in progressive supranuclear palsy (PSP) contain straight filaments composed of pathologically phosphorylated microtubule-associated tau protein and ubiquitin with few PHF (see Jellinger and Bancher, 1992), that distinguish them from NFT in AD, postencephalitic parkinsonism or Guam parkinsonism-dementia complex (Jellinger et al., 1993; Lilienfeld et al., 1994). Other inclusions are either composed of filaments of pathologically phosphorylated neurofilament proteins with ubiquitin, such as Lewy bodies in Parkinson's disease and DLBD (see Jellinger, 1990; Jellinger et al., 1993) or neurofilament and microtubule-associated tau proteins with ubiquitin, as Pick bodies (see Murayama et al., 1990; Lantos, 1992), ubiquitin without other pathological cytoskeletal proteins, as in neuronal and oligodendroglial inclusions in MSA (Papp et al., 1992; Costa and Duyckaerts,

Table 6. Dementing neurodegenerative diseases

1. Lewy body-associated diseases
   a) Parkinson's disease with dementia
   b) Lewy body dementia; diffuse Lewy body disease; senile dementia of Lewy body type; Lewy body variant of Alzheimer disease
2. Neurofibrillary tangle/tau associated disorders
   a) Progressive supranuclear palsy — typical, atypical forms
   b) Parkinson-dementia/ALS complex of Guam
   c) Postencephalitic parkinsonism
3. Other neuronal inclusion disorders
   a) Pick's disease
   b) Corticobasal degeneration
   c) Frontal lobe dementia
4. Oligodendral inclusion disease (Multisystem degeneration/MSA)
   a) Striatonigral degeneration
   b) Olivopontocerebellar atrophy
   c) Shy-Drager syndrome
5. Other neurodegenerative diseases
   a) Huntington's disease
   b) Progressive subcortical gliosis (Neumann's disease)
   c) Thalamic dementia
   d) Joseph disease; pallido-nigral degeneration

1993) and in motoneuron disease (Leigh, 1993) or globular inclusions in corticobasal degeneration (Lang et al., 1994; Wakabayashi et al., 1994). The role of these neuronal and glial changes, considered to represent markers of cytoskeletal dysmetabolism, in the pathogenesis of the relevant degenerative diseases remains to be elucidated. Other dementing neurodegenerative disorders, e.g. Huntington's disease, lack such cellular inclusions (Lantos, 1992; Jellinger et al., 1993).

Parkinson's disease (PD)

Dementia in PD has been related to various brain lesions (Jellinger et al., 1993a; Duyckaerts et al., 1993; Vermersch et al., 1993): damage to subcortico-cortical neuronal systems with cell loss in the medial substantia nigra (mesocortico-limbic dopaminergic deficiency), the noradrenergic (locus ceruleus), serotonergic (dorsal raphe nuclei), and cholinergic systems (nucleus basalis of Meynert), cortical and/or limbic AD pathology, cortical LB pathology, and combinations of cortical and subcortical pathologies. Although neuronal loss in nonadrenergic, serotonergic and cholinergic nuclei is usually more severe in demented than in nondemented PD patients (Jellinger, 1990, 1991; Jellinger et al., 1993a; Duyckaerts et al., 1993), damage to these and other subcortical systems appears not sufficient by itself to produce severe mental decline. By contrast, cortical changes, either AD and/or LB pathology,

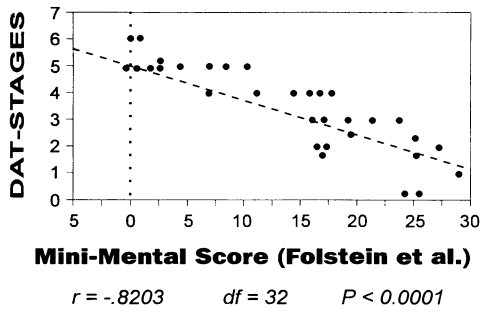

Fig. 3. Correlation between Mini-Mental scores and neuritic Alzheimer stages in 32 consecutive patients with autopsy-confirmed Parkinson's disease (mean age 79.1 yrs)

or both, lead to dementia when of sufficient intensity. In PD brain, neuritic AD pathology reveals a similar pattern and hierarchical topographic spreading from allocortex to isocortical areas with early involvement of the (trans)entorhinal region (Arai et al., 1992; Jellinger et al., 1991; Bancher et al., 1993). Comparative clinico-pathological studies in a cohort of PD patients with graded psychostatus showed similar linear correlation of mental dysfunction with morphological AD staging as seen in aged or AD subjects (Fig. 3). Recent biochemical evidence of predominant deposition of abnormal tau proteins in the entorhinal, temporal and prefrontal cortex suggest a pattern of neuritic pathology somewhat different from AD in some demented PD patients, while the cortex of non-demented PD cases lacks both NFT and pathological tau proteins (Vermersch et al., 1993).

Dementia associated with Lewy bodies

Variously described as Diffuse Lewy body disease (DLBD) (Lennox et al., 1989; Dickson et al., 1992, 1994; Kosaka, 1993; Lippa et al., 1993), LB dementia (Perry et al., 1990, 1993), or LB variant of AD (Hansen et al., 1990, 1993; Förstl et al., 1993) is an increasingly recognized form of primary degenerative dementia that shares both clinical and morphological features with PD and often with AD/DAT, although cognitive defects and psychotic features with hallucinations and confusional states usually overshadow motor deficits and mild extrapyramidal symptoms in late onset cases (Kosaka, 1993; Perry et al., 1990, 1993; Förstl et al., 1993; McKeith et al., 1994). In some recent autopsy series, LBD is suggested to be the second most frequent cause of dementia in the elderly, accounting for 7 to 30% (Dickson et al., 1992; Perry et al., 1993; Table 2). DLBD, histologically featured by the widespread presence of LB in brainstem and cortex – most common in the limbic,

cingulate and insular cortex – and in most cases with neocortical SP often lacking neuritic components but few or no tangles, bears a closer resembance to PD than to AD in its cholinergic neurochemical pathology (Perry et al., 1993). However one-third of the old-onset cases have enough neuritic AD lesions to warrant a diagnosis of AD/DAT (Dickson et al., 1991; Lippa et al., 1993), while in the series of Hansen et al. (1993) 25% of all AD cases were plaque-only AD, 75% of which represented the LB variant of AD. The diagnostic criteria of DLBD depending on the numbers of cortical LB seen in ubiquitin stains (Lennox et al., 1989; Dickson et al., 1992) and the origin of ubiquitin-positive dystrophic neurites in the CA 2/3 region of the hippocampus (Dickson et al., 1994), and the distinction between "pure" DLBD, combined LBD and AD, and AD are still under discussion (Lippa et al., 1993; Harrington et al., 1994).

Progressive supranuclear palsy (PSP)

A sporadic parkinson-like disorder with rigid akinesia, ophthalmoplegia, falls, pseudobulbar palsy and dementia (Litvan and Agid, 1992; Cardoso and Jankovic, 1994; Tolosa et al., 1994), is histologically featured by multisystem neuronal loss and gliosis with widespread NFT composed of straight filaments different from AD-NFT, and widespread tau-immunoreactive neuropil threads and astrocytes. The biochemistry and distribution pattern of NFT is different from that in AD and PD/AD (Vermersch et al., 1994) Dementia in PSP is related to neurofibrillary AD pathology in hippocampus and some neocortical areas (Jellinger and Bancher, 1992; Gearing et al., 1994; Hauw et al., 1994). or to damage to striatofrontal circuits (Pillon et al., 1994).

Pick's disease and lobar atrophies

Accounting for 0.4–2% of all dementing disorders in a large autopsy series (see Jellinger et al., 1990; Table 1), Pick's disease is morphologically featured by frontal and temporal lobe atrophy, neuronal loss with spongiosis and gliosis, intraneuronal 20 nm straight argyrophilic inclusions (Pick bodies) and balloned (Pick) cells composed of tau immunoreactive filaments also reacting with chromogranine A (Murayama et al., 1990; Lassmann et al., 1992; Hauw et al., 1994). and with α β-crystallin that is claimed to define Pick's disease within labor atrophies (Cooper and Mann, 1994). Similar lesions without Pick bodies are seen in *frontal lobe dementia*, a rare presenile form with or without parkinsonism or motoneuron disease (Lantos, 1992; Leigh, 1993; Mann et al., 1993; Brun et al., 1994). Both disorders show severe degeneration of the allocortex that may be difficult to separate from AD (Jellinger,

1994). Hippocampal and neocortical ubiquitin-immunoreactive inclusions in amyotrophic lateral sclerosis (ALS) with dementia differ from Pick bodies in their molecular composition (Leigh, 1993; Mann et al., 1993).

Multisystem atrophies (MSA)

They include a variety of sporadic and autosomal dominant disorders involving several subcortical neuron systems: striatonigral degeneration, olivopontocerebellar atrophy and Shy-Drager syndrome. In addition to neuronal loss and gliosis in striatum, substantia nigra, combined with olivopontocerebellar lesions, they are histologically characterized by argyrophilic, cytoplasmic inclusions in oligodendroglia and/or in neurons (Papp et al., 1992; Lantos, 1992; Costa and Duyckaerts, 1993; Lantos and Papp, 1994).

Corticobasal degeneration (CBD)

A rare disease resembling PSP, with progressive dementia, shows cell loss and gliosis in subcortical nuclei, white matter gliosis associated with swollen achromatic chromatolytic cortical neurons with Pick-like inclusions and subcortical NFT closely resembling those in PSP (Paulus and Selim, 1990; Wakabayashi et al., 1994; Lang et al., 1994)

Huntington's disease

An autosomal dominant neurodegenerative disease transmitted by a gene localized to chromosome 4 and often associated with dementia, morphologically shows diffuse atrophy of the neostriatum (caudate nucleus) and less severe involvement of the neocortex and other subcortical nuclei closely related to the progression of illness and psycho-physical disability (see Vonsattel et al., 1985; Jellinger et al., 1993b). Damage to the entorhinal region mainly affects one deep allocortical layer and is, therefore, different from that in AD/DAT (Braak and Braak, 1992; Jellinger et al., 1994).

Vascular dementias (VD)

Cerebrovascular disease and ischemic brain lesions are considered ba many authors as the second most frequent cause of adult-onset dementias (Jellinger et al., 1990; Chui et al., 1992; Gartia et al., 1992; Roman et al., 1993; Wetterling and Borgis, 1993; Tatemichi et al., 1994; Erkinjunnti and Hachinski, 1993), although the existence of vascular

lesions in the brain of a demented subject may not constitute proof that vascular factors have caused the mental decline, unless other factors are excluded. The combination of cerebrovascular disease (CVD) with degenerative forms of dementia mainly of the AD/DAT type is referred to as mixed type dementia (MIX) or AD + CVD (Roman et al., 1993; Wetterling and Borgis, 1993). It is to be distinguished from AD/DAT with minor CVD lesions. In large autopsy series the incidence of VD ranges from 0–85% (Jellinger et al., 1990; Roman et al., 1993); in Japan, until recently, it represented the most frequent type of autopsy proven dementias with incidence rates of 54 to 65% (Roman et al., 1993; Tatemichi et al., 1993); the incidence of MIX ranges from 1.9 to 27% (Jellinger et al., 1990; Garcia et al., 1992; Roman et al., 1992). The wide incidence ranges may be related to epidemiological, methodological, and classification differences.

Recently, clinical diagnostic criteria for VD and MIX supported by neuroimaging data have been proposed (Chui et al., 1992; Roman et al., 1993; Wetterling and Borgis, 1993; Tatemichi et al., 1993; Erkinjunnti and Hachinski, 1993) but need to be proven by prospective clinico-pathological studies. (Lopez et al., 1994) VD constitutes a multifactorial disorder related to a variety of morphologic lesions that are summarized in Table 7.

In a personal consecutive autopsy cohort of demented aged persons, VD with no or only age-related AD lesions accounted for

Table 7. Major morphological types of vascular dementia (modified according to Garcia and Brown, 1992; Roman et al., 1993)

1. Classical multiinfarct encephalopathy (MIE)
   Multiple large (sub-/territorial) infarcts in cortex and white matter/basal ganglia in territories of large cerebral arteries, particularly MCA, MCA + PCA; frequently bilaterally of involving both hemispheres
2. Strategic infarct dementia (SID)
   Small or medium-sized infarcts/ischemic scars in functionally important brain regions: thalamus (Art. thalamoperforata of the PCA); hippocampus (PCA), angulate gyrus and basal forebrain (ACA), bilaterally or dominant hemisphere
3. Microangiopathic (small vessel infarct) dementia (SMVA)
   a) Subcortical arteriosclerotic encephalopathy Binswanger (SAE) Subcortical leukoence-phalopathy
      Multiple small infarcts in basal ganglia and hemispheral white matter with preservation of cerebral cortex
   b) Multilacunar state
      Multiple microinfarcts (up to 1.5 cm ϕ); hemorrhagic or infact scars in basal ganglia, hemisphereal white matter, pontine basis
      Multiple cortico-subcortical microinfarctions (mixed encephalopathies)
   c) Granular cortical atrophy
      Multiple small ischemic or infarction scars within the border zones between ACA and MCA in one or both hemispheres

12.9%; MIX (AD + CVD) 5.5% of the total as compared to 15.5% of AD/DAT cases with minor vascular lesions (Table 1). The mean age at death of VD patients (78.2 ± 8.3 yrs) did not differ considerably from that of the total dementia series (79.5 ± 6.7 years). In the recent group of 50 autopsies of demented elderly, VE accounted for only 8% and MIX for 6% as compared to 10% AD with minor vascular lesions (Table 2).

Morphological subtypes of VD were as follows (Fig. 4):

1. Microangiopathic (small vessel infarct) dementia SMVD was seen in 41.3%, the majority as Binswanger type subcortical arteriosclerotic encephalopathy (SAE) – 38.7% –, with less frequent multilacunar state and multiple cortico-subcortical microinfarcts (mixed vascular type in 9%, and granular cortical atrophy (2.6%). 2. Classical *multiinfarct encephalopathy* (MIE) – 39.4% – with large infarcts in both (66%), left (23%) or right hemispheres (11%) showing (sub)territorial infarcts in cerebral hemispheres and basal ganglia (77%) involving the following vascular territories (Fig. 5): MCA in 42%, MCA + PCA (fronto-temporo-parieto-occipital), often with involvement of striatum and/or thalamus, in 41%, ACA + MCA + PCA in 6%, ACA + MCA 3%, and ACA + PCA in 2%; in 23% additional lesions involved the cerebellum and/or brainstem. *3. Strategic infarct dementia (SID)* in 19.3%, with involvement of both (73%) or the left hemisphere (27%) by multiple small infarcts in

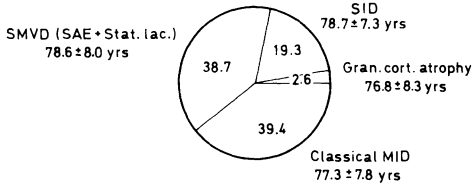

Fig. 4. Percent incidence of major morphological subtypes of vascular dementia in 155 consecutive autopsy cases (age 78.2 ± 8.3 yrs)

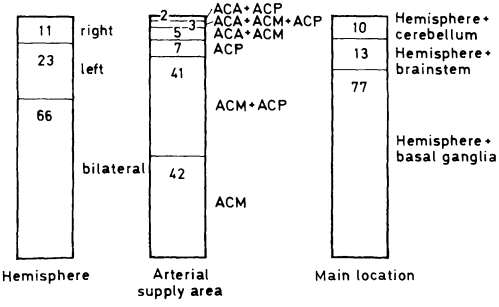

Fig. 5. Morphological lesion patterns in multi-infarct encephalopathies (61 consecutive autopsy cases; multiple infarcts 97%)

(medial) thalamic nucleus, often bilaterally (33%), thalamus + striatum (27%), thalamus + brainstem (6%), thalamus and hippocampus (13%) with brainstem (18) or other combinations in 3% (Fig. 6).

MIX type dementias include a combination of AD/DAT with MIE in 70%, SID (17%) and SMVE (13%) (Fig. 7). Not included were cases of AD/DAT with small cerebrovascular lesions (<50 ml total volume) accounting for 15.5% of all dementia cases and 19.8% of all AD/DAT subjects. The lesion patterns of the combination of AD + MIE were similar to those in pure MIE (43% bilateral, 33% left, 24% right hemisphere), but with preponderance of infarcts cerebral hemispheres and basal ganglia (85%), in the MCA region (56%) towards MCA + PCA (28%), ACA + MCA, PCA + ACA + MCA (7% each) and ACA bilateral (2%) with additional lesions in cerebellum and/or brainstem in 15% (Fig. 8).

The combination of AD + SID showed infarcts bilaterally or in the left hemisphere (73 and 27%, respectively), mainly in the (medial) thalamus either isolated (18%) or in combination with lesions in

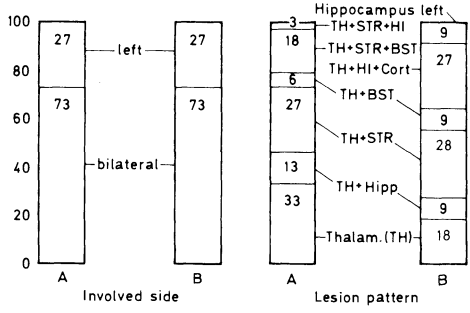

Fig. 6. Morphological lesion patterns of the strategic infarct dementia type. A. Vascular encephalopathies (n = 30) B. Mixed-type dementia (n = 11)

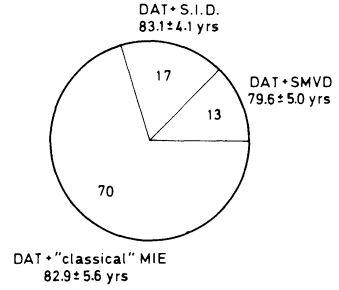

Fig. 7. Incidence of mixed type dementia (Alzheimer type combined with vascular pathologies) in 66 autopsy proven cases of dementia (age 83.2 ± 5.5 yrs)

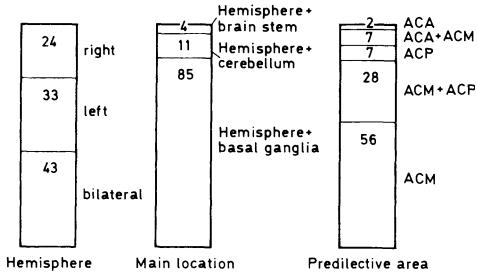

Fig. 8. Morphologic lesion pattern of mixed type dementia (MIX) in 46 autopsy cases (%; n = 46; multiple infarcts: 96%)

hippocampus (9%) and cortex (30%), striatum (28%) and brainstem (19%) (Fig. 6). A combination of AD + PSVE was seen in 13% of MIX cases in which a distinction between Binswanger type SAE and leukoencephalopathy in AD or with amyloid angiopathy (Gray et al., 1985) was attempted. The mean age at death in the MIX cases (83.2 ± 5.5 years) did not differ considerably from that in "pure" AD/DAT cases and AD with vascular lesions (82.1 or 81.6 ± 5.5 years).

The retrospectively evaluated coincidence rates between clinical and post-mortem diagnosis were 84% for AD/DAT and 90% for the VD group which is in accordance with data from the literature (Jellinger et al., 1990; Roman et al., 1993), whereas only in 6% of the MIX cases a correct clinical diagnosis had been made, the most frequent diagnosis being VD, which emphasizes the difficulties in its distinction from other dementias (Fig. 9). These data confirm the presence of cerebrovascular lesions in about 20% of all autopsy cases of adult-onset dementia; 12.9% of our group represented "pure" vascular forms without accompanying AD pathology, and 5.5% a combination of DAT and CVD in which the latter lesions may dominate the clinical picture. In further 15.5% with

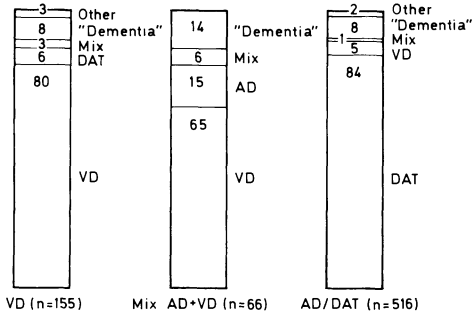

Fig. 9. Coincidence rates of clinical and post mortem diagnoses in 737 consecutive autopsy cases of aged demented individuals (%)

massive AD pathology the presence of additional minor cerebrovascular lesions may be without clinical relevance. 2.3% of AD/DAT brains in our series revealed medium to large-sized hemorrhages related to amyloid angiopathy (see Table 1). In VD, recent positron emission tomography (PET) studies showed that the severity of dementia was significantly related to the volume of metabolically impaired tissue (Mielke et al., 1992). Synaptophysin immunoreactivity of the cortical neuropil in vascular dementia of the Spatz-Lindenberg type (granular cortical atrophy) was as much reduced as in AD (Zhan et al., 1993).

Transmissible spongiform encephalopathies (Prion diseases)

The transmissible spongiform encephalopathies or Prion diseases are a group of 4 human and 4 animal diseases that are caused by – as yet – unclassified transmissible pathogens (Table 8). All these diseases have in common an unusually long incubation period ranging in the order of months or even years and a histopathology that lacks any signs of an immune response from the host (see Prusiner, 1993a, b; Brown, 1994). The pathology is strictly restricted to the brain and consists of spongiform changes of the neuropil, severe neuronal loss, astrogliosis and multicentric amyloid plaques (Kretzschmar, 1993; Watanabe and Duchen, 1993; Brown, 1994). In contrast to SP in AD/DAT, amyloid deposits in these conditions are made of a protease-resistant isoform of a 30 to 35-kDa sialoglycoprotein termed prion protein (PrP). Extracts of the brains of infected individuals contain so-called scrapie-associated filaments that have the characteristics of amyloid and are composed of PrP. These fractions are highly infective in transmitting the disease to laboratory animals (Gajdusek, 1991; Prusiner, 1993a, b; Liberski, 1993). The most favoured hypothesis states that the infectious pathogen is a

Table 8. Prion diseases[1]

Disease	Natural host
Scrapie	sheep, goats
Transmissible mink encephalopathy (TME)	mink
Chronic wasting disease (CWD)	mule deer, elk
Bovine spongiform encephalopathy (BSE)	cattle
Kuru	humans – fore
Creutzfeldt-Jakob disease (CJD)	humans
Gerstmann-Sträussler-Scheinker syndrome (GSS)	humans
Fatal familial insomnia	humans

[1] Alternative terminologies are: slow virus infections, subacute transmissible spongiform encephalopathies, unconventional slow virus diseases, infections cerebral amyloidosis

proteinaceous agent (PrP) encoded by a normal cellular gene (Gajdusek, 1991; Prusiner, 1993a, b; Brown, 1994). PrP may potentially generate disease by either binding to DNA to induce its own synthesis or by self-replicating by as yet unknown mechanisms. In essential, two of the human prion diseases are causes of dementia, i.e. Creutzfeldt-Jakob disease (CJD) and Gerstmann-Sträussler-Scheinker syndrome (GSS). Whereas CJD is essentially a sporadic illness occuring world-wide, GSS is a unique example of a disease that is both genetic and infectious. Both GSS, fatal familial insomnia (Medori et al., 1992) and the rare familial form of CJD are inherited in an autosomal dominant pattern, and in some families single point mutations in the PrP gene have been reported (Prusiner, 1993a, b; Brown, 1994). CJD is clinically characterized by subacute dementia, pyramidal, extrapyramidal and cerebellar signs, occasional abnormal eye movements (Grant et al., 1993), and myoclonus. Therefore, it might be confused with rapidly progressive AD in which myoclonus may also occur (Lantos, 1992; Felgenhauer et al., 1993), but rare coexistence of sporadic CJD and AD has been reported (Liberski, 1993; Brown, 1994). GSS is an autosomal dominant disorder clinically featured by progressive ataxia, nystagmus and other eye movement disorders (Grant et al., 1993). The most prominent histological feature is the presence of numerous amyloid plaques in cerebellum, cerebral cortex and basal ganglia that may or may not be associated with spongiform changes (Kretzschmar, 1993; Brown, 1994).

Normal pressure hydrocephalus

A rare cause of dementia in advanced age is chronic hydrocephalus associated with a clinical triad consisting of progressive dementia, gait disturbance and urinary incontinence caused by an imbalance between CSF production and resorption without obstructive changes in the CSF pathways (Dippel and Habbema, 1993). The morphology of this treatable type of dementia is non-specific including fibrosis of the meninges and the choroid plexus, periventricular gliosis, etc. (Miller and Adams, 1992), while in some cases, biopsy reveals AD, in which brain atrophy is cause of an e vacuo hydrocephalus.

Other organic brain diseases

A large number of organic brain diseases other than the entities cited above can lead to mental decline in advanced age (Tomlinson, 1992; Tatemichi et al., 1994). These conditions include posttraumatic brain damage such as boxer's encephalopathy or dementia pugilistica featuring part of the histopathology of AD/DAT including multiple A β

deposits (Roberts et al., 1994). Among other causes of dementia are chronic inflammatory conditions, such as demyelinating conditions (multiple sclerosis) (Fontaine et al., 1994) or the sequelae of herpes simplex virus encephalitis particularly involving the limbic temporal lobes (Kennedy et al., 1988; Schmidbauer et al., 1991). In aged subjects, the differential diagnosis versus AD may be difficult, but is possible by modern immunological methods including polymerase chain reaction (Pohl-Koppe et al., 1992; Puchhammer-Stöckl et al., 1993). Other inflammatory CNS diseases that should be clinically distinguished from AD are chronic inflammatory meningoencephalitis associated with Sjögren's syndrome (99) and paraneoplastic limbic encephalitis (Vollmer et al., 1993; Sutton et al., 1993).

Among the long list of central nervous system disorders associated with dementing syndromes, there is increasing incidence of specific disorders associated with alcoholism, often presenting as Korsakoff-Wernicke's syndrome (Tomlinson, 1992; Liszka et al., 1993). It is clinically featured by the triad of mental disturbances, eye movement disorders and ataxia. Morphologic studies reveal brain atrophy and incomplete, partially hemorrhagic tissue necroses in mammillary bodies, the walls of the third ventricle, and in brainstem tegmentum. Wernicke's encephalopathy is most frequently found in alcoholics, but also may occur in all kinds of malnutrition and in acquired immunodeficiency syndrome (AIDS) (Liszka et al., 1993).

Finally, dementia may be a sequel of cerebral ischemia or hypoxia (Garcia and Brown, 1992) or be the result of metabolic and endocrine disturbances (Tomlinson, 1992; Tatemichi et al., 1994). The morphological brain changes vary with the illness involved. Many patients with progressive dementia fall into one or other of the established categories, but nosologic overlap is common, and, occasionally, the abnormalities associated with dementia are unclassified. Due to recent progress in neuroscience, however, the numbers of demented cases undiagnosed after full clinical and neuropathological examination, already a minimal proportion (Tables 1 and 2), may well become insignificant in the near future. Further elucidation of the molecular origins of dementia being a major subject of current neuroscience research will promote early diagnosis and more successful treatment strategies for dementing processes.

References

American Psychiatric Association. Committee on Nomenclature (1987) Diagnostic and statistical manual of mental disorders, 3nd edn, revised. American Psychiatric Association, Washington DC

Arai H, Schmidt ML, Lee VM-Y, et al (1992) Epitope analysis of senile plaque components in the hippocampus of patients with Parkinson's disease. Neurology 42:1315–1322

Armstrong RA, Myers D, Smith CUM (1993) The spatial patterns of plaques and tangles in Alzheimer's diseases do not support the "cascade hypothesis". Dementia 4:16–20

Arnold SE, Kumar A (1993) Reversible dementias. Med Clin North Am 77:215–130

Arriagada PV, Growdon JH, Hedley-Whyte T, et al (1992) Neurofibrillary tangles but not senile plaques parallel duration and severity of Alzheimer's disease. Neurology 42:631–639

Arriagada PV, Marzhoff K, Hyman BT (1993) Distribution of Alzheimer-type pathologic changes in nondemented elderly individuals matches the pattern in Alzheimer's disease. Neurology 42:1681–1688

Bachmann DL, Wolf PA, Linn RT, Knoefel JE, Cobb JL, Belanger AJ, White LR, D'Agostino RB (1993) Incidence of dementia and probable Alzheimer's disease in a general population. Ann Neurol 43:515–519

Bancher C, Grundke-Iqbal K, Fried VA, Smith HT, Wisniewski HM (1991) Abnormal phosphorylation of tau precedes ubiquination of neurofibrillary pathology of Alzheimer's disease. Brain Res 539:11–18

Bancher C, Braak H, Fischer P, Jellinger K (1993) Neuropathological staging of Alzheimer lesions and intellectual status in Alzheimer's and Parkinson's disease. Neurosci Lett 162:179–182

Bancher C, Jellinger KA (1994) Neurofibrillary tangle predominant form of Alzheimer's dementia: a rare subtype in very old subjects. Acta Neuro-pathol 88 (in press)

Bancher C, Paulus W, Paukner K, Jellinger K (1993) A questionnaire on the post-mortem diagnosis of Alzheimer's disease: evidence for lack of consensus between practicing neuropathologists. Clin Neuropathol 12:233

Bennett DA, Cochran EJ, Saper CB, et al (1993) Pathological changes in frontal cortex from biopsy to autopsy in Alzheimer's disease. Neurobiol Aging 14:589–596

Benzing WC, Ikonomovic MD, Brady DB, Mufson AJ, Armstrong DM (1993a) Evidence that transmitter-containing dystrophic neurites precede those containing paired helical filaments within senile plaques in the entrohinal cortex of nondemented elderly and Alzheimer's disease patients. Brain Res 619:55–68

Benzing WC, Ikonomovic MD, Brady DB, Mufson AJ, Armstrong DM (1993b) Evidence that transmitter-containing dystrophic neurites precede paired helical filament and Alz-50 formation within senile plaques in the amygdala of nondemented elderly and patients with Alzheimer's disease. Brain Res 619:55–68

Berg L, McKee DW, Miller JP, Barty J, Morris JC (1993) Neuropathological indices of Alzheimer's disease in demented and nondemented persons aged 80 years and older. Arch Neurol 50:34–358

Bouras C, Hof PR, Morrision JH (1993) Neurofibrillary tangle densities in the hippocampal formation in a non-demented population define subgroups of patients with differential early pathologic changes. Neurosci Lett 153:131–135

Braak H, Braak E (1991) Neuropathological staging of Alzheimer-related changes. Acta Neuropathol 82:239–259

Braak H, Braak E (1992) The human entorhinal cortex: normal morphology and lamina-specific pathology in various diseases. Neurosci Res 15:6–31

Braak H, Braak E (1994) Pathology of Alzheimer's disease. In: Calne DB (ed) Neurodegenerative disease. Saunders, Philadelphia, pp 585–613

Braak H, Duyckaerts C, Braak E, Piette F (1993) Neuropathological staging of Alzheimer-related changes correlates with psychometrically assessed intellectual status. In: Corain B, et al (eds) Alzheimer's disease. Advances in clinical and basic research. Wiley, Chichester, pp 131–137

Braak H, Braak E, Mandelkow EM (1994) A sequence of cytoskeleton changes related to the formation of neurofibrillary tangles and neuropil threads. Acta Neuropathol 87:554–567

Brown P (1994) Transmissible human spongiform encephalopathy (infectious cerebral amyloidosis); Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker syndrome, and Kuru. In: Calne DB (ed) Neurodegenerative diseases. Saunders, Philadelphia, pp 839–876

Brun A, Englund B, Gustavson U, et al (1994) Clinical and neuropathological criteria for frontotemporal dementia. J Neurol Neurosurg Psychiatry 57:416–418

Cardoso F, Jankovic J (1994) Progressive supranuclear palsy. In: Calne DB (ed) Neurodegenerative diseases. Saunders, Philadelphia, pp 769–786

Caselli RJ, Scheithauer BW, O'Duffy JD, et al (1993) Chronic inflammatory meningoencephalitis should not be mistaken for Alzheimer's disease. Mayo Clin Proc 68:846–855

Chui HC, Tierney M, Zarow C, Lewis A, Sobel E, Perlmutter LS (1993) Neuropathologic diagnosis of Alzheimer disease: interrater reliability in the assessment of senile plaques and neurofibrillary tangles. Alzheimer Dis Assoc Disord 7:48–54

Chui HC, Victoroff JI, Margolin D, et al (1992) Criteria for the diagnosis of ischemic vascular dementia proposed by the State of California Alzheimer's disease diagnostic and treatment centers. Neurology 42:473–480

Cooper PN, Mann DMA (1994) Alpha B-crystallin-immunostaining defines Pick's disease within lobar atrophy (abstr.) Ann Neurol 36:309

Coria F, Moreno A, Rubio I, Garcia MA, Morato E, Major Jr F (1993) The cellular pathology associated with Alzheimer β-amyloid deposits in non-demented aged individuals. Neuropathol Appl Neurobiol 19:261–268

Costa C, Duyckaerts C (1993) Oligodendroglial and neuronal inclusions in multiple system atrophy. Curr Opin Neurol 6:865–871

Crystal HA, Dickson DW, Sliwinski MJ, Lipton RB, Grober E, Marks-Nelson H, Antis P (1993) Pathological markers associated with normal aging and dementia in the elderly. Ann Neurol 34:566–573

DeKosky ST, Scheff SW (1990) Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 27:457–464

DeKosky ST, Harbaugh RE, Schmitt FA, et al (1992) Cortical biopsy in Alzheimer's disease: diagnostic accuracy and neurochemical. Neuropathological and cognitive correlations. Ann Neurol 32:625–632

De Lacoste M-C, White C III (1993) The role of cortical connectivity in Alzheimer's disease pathogenesis; a review and model system. Neurobiol Aging 14:1–16

Delacourte A (1993) The pathophysiological basis of Alzheimer's disease. Therapie 48:177–183

Delaere P, Duyckaerts C, He Y, Piette F, Hauw JJ (1991) Subtypes and differential laminar distributions of βA4 deposits in Alzheimer's disease: relationship with the intellectual status of 26 cases. Acta Neuropathol 81:328–335

Delaere P, He Y, Fayet G, Duyckaerts C, Hauw JJ (1993) β-A4 deposits are

constant in the brain of the oldest old. Neurobiol Aging 14:191–194
Dickson DW, Crystal HA, Mattiace LA, Masur DM, Blau AD, Davies P, Yen SH, Aronson MK (1991) Identification of normal and pathological aging in prospectively studied nondemented elderly humans. Neurobiol Aging 13:179–189
Dickson DW, Schmidt ML, Lee VM-Y, et al (1994) Immunoreactive profile of hippocampal CA 2/3 neurites in Diffuse Lewy body disease. Acta Neuropathol 87:269–276
Dickson DW, Wu E, Crystal HA, Mattiace LA, Yen S-H, Davies P (1992) Alzheimer and age-related pathology in diffuse Lewy body disease. In: Boller F, et al (eds) Heterogeneity of Alzheimer's disease. Springer, Berlin Heidelberg New York Tokyo, pp 168–186
Dippel DWJ, Habbema JDF (1993) Probalistic diagnosis of normal pressure hydrocephalus and other treatable cerebral lesions in dementia. J Neurol Sci 119:123–133
Duyckaerts C, Gaspar P, Costa C, Bonnet AM, Hauw JJ (1993) Dementia in Parkinson's disease. Morphometric data. Adv Neurol 60:447–455
Erkinjunnti T, Hachinski VC (1993) Rethinking vascular dementia. Cerebrovasc Disord 3:3–23
Esiri MM, Pearson RCA, Steele JE, Bowen DM, Powell TPS (1990) A quantitative study of the neurofibrillary tangles and the choline acetyltransferase activity in the cerebral cortex and the amygdala in Alzheimer's disease. J Neurol Neurosurg Psychiatry 53:161–165
Evans DA, Funkenstein HH, Albert MS, et al (1989) Prevalence of Alzheimer's disease in a community population of older persons; higher than previsously reported. JAMA 262:2551–2556
Felgenhauer K, Weber T, Poser S (1993) Early clinical and laboratory diagnosis of Creutzfeldt-Jakob disease. Dev Biol Stand 80:125–130
Folstein MF, Folstein SE, McHugh PR (1975) "Mini-Mental State": a practical method for grading the cognitive state of patients for the clinician. J Psychiatry Res 12:189–198
Fontaine B, Seilhean D, Tourban A, Daumas-Duport C, Duyckaerts C, Benoit N, Devaux B, Hauw JJ, Rancurel G, Lyon-Caen O (1994) Dementia in two histologically confirmed cases of multiple sclerosis: one case with isolated dementia and one case associated with psychiatrc symptoms. J Neurol Neurosurg Psychiatry 57:353–359
Förstl H, Burns A, Luther P, et al (1993) The Lewy body variant of Alzheimer's disease. Br J Psychiatry 162:385–392
Förstl H, Sattel H, Bahro M (1993) Alzheimer's disease; clinical features. Int Rev Psychiat 5:327–349
Gajdusek DC (1991) The transmissible amyloidoese. Eur J Epidemiol 7:567–577
Garcia JH, Brown GG (1992) Vascular dementia: neuropathologic alterations and metabolic brain changes. J Neurol Sci 109:121–131
Gearing M, Olson DA, Watte RL, Mirra SS (1994) Progressive supranuclear palsy: neuropathologic and clinical heterogeneity. Neurology 44:1015–1024
Goedert M (1993) Tau protein and the neurofibrillary pathology of Alzheimer's disease. Trends Neurosci 16:460–465
Grant MP, Cohen M, Peterson RR, et al (1993) Abnormal eye movements in Creutzfeldt-Jakob disease. Ann Neurol 34:192–197
Gray F, Dubas F, Roullet E, Escourolle R (1985) Leukoencephalopathy in diffuse hemorrhagic cerebral amyloid angiopathy. Ann Neurol 18:54–59

Hansen LA, Masliah E, Galasko D, Terry RD (1993) Plaque-only Alzheimer disease is usually the Lewy body variant and vice versa. J Neuropathol Exp Neurol 52:648–654

Hansen L, Salmon D, Galasko D, et al (1990) Lewy body variant of Alzheimer's disease: a clinical and pathological entity. Neurology 40:1–8

Harrington CR, Perry RH, Perry BK, et al (1994) Senile dementia of Lewy body type and Alzheimer type are biochemically distinct in terms of paired helical filaments and hyperphosphorylated tau protein. Dementia 5:215–228

Hauw JJ, Daniel S, Dickson D, et al (1994) The preliminary NINDS neuropathologic criteria for Steele-Richardson-Olszewski syndrome (progressive supranuclear palsy). Neurology (in press)

Hof PR, Morrison JH (1994) The cellular basis of cortical disconnection in Alzheimer disease and related dementing conditions. In: Terry RD, Katzman R, Bick KL (eds) Alzheimer disease. Raven Press, New York, pp 197–229

Hofmann A (1993) Epidemiology of Alzheimer's disease. In: Wolters ECh, Scheltens P (eds) Alzheimer's disease: back to the future. Vrije Universiteit, Amsterdam, pp 9–20

Honer WG, Dickson DW, Gleeson J, Davies P (1992) Regional synaptic pathology in Alzheimer's disease. Neurobiol Aging 13:375–382

Hubbard BM, Fenton GW, Anderson JM (1990) A quantitative histological study of early clinical and preclinical Alzheimer's disease. Neuropathol Appl Neurobiol 16:111–121

Hyman BT, Tanzi RE (1992) Amyloid, dementia and Alzheimer's disease. Curr Opin Neurol Neurosurg 5:88–93

Hyman BT, Van Hoesen GW, Damasio AR (1990) Memory-related neuronal systems in Alzheimer's disease: an anatomic study. Neurology 40:1721–1730

Jellinger K (1990) New developments in the pathology of Parkinson's disease. Adv Neurol 53:1–16

Jellinger K (1991) Pathology of Parkinson's disease. Changes other than the nigrostriatal pathway. Mol Chem Neuropathol 14:153–197

Jellinger KA (1993) Morphology of the aging brain and relation to Alzheimer's disease. In: Calne DB, et al (eds) Advances in research on neurodegeneration, I. Birkhäuser, Boston, pp 107–137

Jellinger KA (1994) Quantitative neuropathologic analysis of Pick's disease. Acta Neuropathol 87:223–224

Jellinger K, Bancher C (1992) Neuropathology. In: Litvan I, Agid Y (eds) Progressive supranuclear palsy. Clinical and research approaches. Oxford University Press, New York, pp 44–88

Jellinger KA, Bancher C, Fischer P (1993a) Neuropathological correlates of mental dysfunction in Parkinson's disease. In: Wolters AC, Scheltens P (eds) Mental dysfunction in Parkinson's disease. Vrije Universiteit, Amsterdam, pp 141–161

Jellinger K, Braak H, Braak E, Fischer P (1991) Alzheimer lesions in the entorhinal region and isocortex in Parkinson's and Alzheimer's diseases. Ann NY Acad Sci 640:203–209

Jellinger K, Danielczyk W, Fischer P, Gabriel E (1990) Clinicopathological analysis of dementia disorders in the elderly. J Neurol Sci 95:239–258

Jellinger KA, Lantos PL, Mehraein P (1993b) Pathological assessment of movement disorders: requirements for documentation in brain banks. J Neural Transm [Suppl] 39:173–184

Jones RSG (1993) Entorhinal-hippocampal connections: a speculative view of

their function. Trends Neurosci 16:58–64
Katzman R, Kawan C (1994) The epidemiology of dementia and Alzheimer disease. In: Terry RD, Katzman R, Bick KL (eds) Alzheimer disease. Raven Press, New York, pp 105–122
Kazee AM, Eskin TA, Lapham LW, Gabriel RR, McDaniel RD, Hamill RW (1993) Clinicopathologic correlates in Alzheimer's disease – assessment of clinical and pathologic diagnostic criteria. Alzheimer Dis Assoc Disord 7:152–164
Kennedy PGE, Adams JH, Graham DI, Celements GB (1988) A clinico-pathological study of herpes simplex encephalitis. Neuropathol Appl Neurobiol 14:395–415
Khachaturian ZS (1985) Diagnosis of Alzheimer's disease. Arch Neurol 42:1097–1105
Killiany RJ, Moss MB, Albert MS, Sandor T, Tieman J, Jolesz F (1993) Temporal lobe regions on magnetic resonance imaging identify patients with early Alzheimer's disease. Arch Neurol 50:949–954
Kosaka K (1993) Dementia and neuropathology in Lewy body disease. Adv Neurol 60:456–463
Kretzschmar HA (1993) Neuropathology of human Prion diseases (spongiform encephalopathies). Dev Biol Stand 80:71–90
Lantos PL (1992) Neuropathology of unusual dementias: an overview. In: Rossor MN (ed) Unusual dementias. Baillière's Clin Neurol 1 (3):485–516
Lantos PL, Papp HI (1994) Cellular pathology of multiple system atrophy; a review. J Neurol Neurosurg Psychiatry 57:129–133
Lang AE, Riley DE, Bergeron C (1994) Cortico-basal ganglionic degeneration. In: Calne DB (ed) Neurodegenerative diseases. Saunders, Philadelphia, pp 877–894
Lassmann H, Bancher C, Breitschopf H, et al (1994) Cell death in Alzheimer's disease evaluated by DNA fractionation in situ. Acta Neuropathol (in press)
Lassmann H, Weiler R, Fischer P, Bancher C, Jellinger K, et al (1992) Synaptic pathology in Alzheimer's disease: immunological data for markers of synaptic and large dense core vesicles. Neuroscience 46:1–8
Lauter H, Kurz A, Haupt B, Romero B, Zimmer R (1990) Clinical diagnosis of Alzheimer's disease: DSM-III-R, ICD-10 – what else? In: Maurer K, Riederer P, Beckmann H (eds) Alzheimer's disease. Epidemiology, neuropathology, neurochemistry and clinics. Springer, Wien New York, pp 365–371
Lehericy S, Hirsch EC, Cervera-Pierot P, et al (1993) Heterogeneity and selectivity of the degenerations of cholinergic neurons in the basal forebrain of patients with Alzheimer's disease. J Comp Neurol 330:15–31
Leigh PN (1993) Pathogenic mechanisms in amyotrophic lateral sclerosis and other motor neuron disorders. In: Calne DB, et al (eds) Advances in research on neurodegeneration. Birkhäuser, Boston, pp 163–182
Lennox G, Lowe J, Landon M, et al (1989) Diffuse Lewy body disease: correlative neuropathology using anti-ubiquitin immunocytochemistry. J Neurol Neurosurg Psychiatry 52:1236–1247
Liberski PP (1993) The enigma of slow viruses. Facts and artefacts. Arch Virol [Suppl] 6:1–277
Lilienfeld DC, Perl DP, Olanow CW (1994) Guam neurodegeneration. In: Calne DB (ed) Neurodegenerative diseases. Saunders, Philadelphia, pp 895–908
Lippa CF, Smith TW, Swearer JM (1993) Alzheimer's disease and Lewy body disease: a comparative clinicopathological study. Ann Neurol 35:81–88

Liszka U, Drlicek M, Hitzenberger P, et al (1993) Wernicke-Enzephalopathie – Klinik und Neuropathologie. Neuropsychiatrie 7:129–132

Litvan I, Agid Y (eds) (1992) Progressive supranuclear palsy. Clinical and research approaches. Oxford University Press, New York

Lopez Ol, Larumba MR, Becker JT, Rezek D, Rosen J, Klunk W, Dekosky ST (1994) Reliability of NINDS-AIREN clinical criteria for the diagnosis of vascular dementia. Neurology 44:1240–1245

MacKenzie IRA (1994) Senile plaques do not progressively accumulate with normal aging. Acta Neuropathol 87:520–525

Mann DMA (1994) Pathological correlates of dementia in Alzheimer's disease. Neurobiol Aging 15:357–360

Mann DMA, South PW, Snowden JP, Neary D (1993) Dementia of frontal lobe type: neuropathology and immunohistochemistry. J Neurol Neurosurg Psychiatry 56:605–614

Markesberry WR, Wang HZ, Kowau NW, Kosik KS, McKee AC (1993) Morphometric image analysis of neuropil threads in Alzheimer's disease. Neurobiol Aging 14:303–307

Masliah E, Ellisman M, Carragher M, et al (1992) Three dimensional analysis of the relationship between synaptic pathology and neuropil threads in Alzheimer's disease. J Neuropathol Exp Neurol 51:404–414

Masliah E, Honer WG, Mallory M, et al (1993) Topographical distribution of synaptic-associated proteins in the neuritic plaques of Alzheimer's disease hippocampus. Acta Neuropathol 87:135–142

Masliah E, Mallory M, Hansen L, DeTeresa BS, Terry RD (1993) Quantitative synaptic alterations in the human neocortex during normal aging. Neurology 43:192–197

Masliah E, Mallory M, Deerinck T, et al (1993) Re-evaluation of the structural organization of neuritic plaques in Alzheimer's disease. J Neuropathol Exp Neurol 52:619–631

Mattson MP, Barger SW, Cheng B, et al (1993) β-amyloid precursor protein metabolites and loss of neuronal Ca^{2+} homeostasis in Alzheimer's disease. Trends Neurosci 16:409–414

McKee AC, KosiK KS, Kowall NW (1992) Neuritic pathology and dementia in Alzheimer's disease. Ann Neurol 30:156–165

McKeith IG, Fairbairn AF, Bothwell RA, Moore PB, Ferrier IN, Thompson P, Perry RH (1994) An evaluation of the predictive validity and inter-rater reliability of clincial diagnostic criteria for senile dementia of Lewy body type. Neurology 44:5

McKeel DW, Ball MJ, Price JL, Smith DS, Miller JP, Berg L, Morris JC (1993) Interlaboratory histopathologic assessment of Alzheimer neuropathology — different methodologies yield comparable diagnostic results. Alzheimer Dis Assoc Disord 7:136–151

McKhann GD, Drachmann DA, Folstein MF, et al (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939–944

Medori R, Tritschler HJ, LeBlanc A, et al (1992) Fatal familial insomnia, a prion disease with a mutation at codon 178 of the protein gene. N Engl J Med 326:444–449

Mena R, Wischik CM, Novak M, Milstein C, Cuello AC (1991) A progressive deposition of paired helical filaments (PHF) in the brain characterizes the evolution of dementia in Alzheimer's disease. J Neuropathol Exp Neurol

50:474–490
Mielke R, Herholz K, Grond M, Kessler J, Heiss WD (1992) Severity of vascular dementia is related to volume of metabolically impaired tissue. Arch Neurol 40:909–913
Miller JD, Adams JH (1992) The pathophysiology of raised intracranial pressure. In: Adams JH, Duchen LW (eds) Greenfield's neuropathology, 5th ed. E Arnold, London, pp 69–105
Mirra SS, Hart MN, Terry RD (1993) Making the diagnosis of Alzheimer's disease. Arch Pathol Lab Med 117:132–144
Mirra SS, Gearing M, McKeel DW, Crain BJ, Hughes JP, Van Belle G, Heyman A (1994) Interlaboratory comparison of neuropathology assessments in Alzheimer's disease: a study of the Consortium to Establish a Registry for Alzheimer's Disease (CERAD). J Neuropathol Exp Neurol 53:303–315
Morris JC, McKeel DW Jr, Storandt M, et al (1991) Very mild Alzheimer's disease: informant-based clinical, psychometric, and pathological distinction from normal aging. Neurology 41:469–478
Mulhaupt G, Masters CL, Beyreuther K (1993) A molecular approach to Alzheimer's disease. Biol Chem Hoppe Seyler 374:1–8
Mullan H, Crawford F (1993) Genetic and molecular advances in Alzheimer's disease. Trends Neurosci 16:398–403
Murayama S, Mori H, Ihaia Y, Tomonaga M (1990) Immunocytochemical and ultrastructural studies of Pick's disease. Ann Neurol 27:394–405
Papp MI, Kahn JE, Lantos PL (1992) Glial cytoplasmic inclusions in the CNS of patients with multiple system atrophy (striatonigral degeneration, olivo-pontocerebellar atrophy and Shy-Drager syndrome). J Neurol Sci 107:172–182
Pappola MA, Omar RA, Vinters HV (1991) Image analysis microspectoscopy shows that neurons participate in the genesis of a subset of early primitive (diffuse) senile plaques. Am J Pathol 139:599–607
Paulus W, Bancher C, Jellinger K (1992) Interrater reliability in the neuropathologic diagnosis of Alzheimer's disease. Neurology 42:329–332
Paulus W, Selim M (1990) Corticonigral degeneration with neuronal achromasia and basal neurofibrillary tangles. Acta Neuropathol 81:89–94
Perry EK, Irving D, Kerwin JM, et al (1993) Cholinergic transmitter and neurotrophic activities in Lewy body dementia: similarity to Parkinson's and distinction from Alzheimer's disease. Alzheimer Dis Assoc Disord 7:69–79
Perry RH, Irving D, Blessed G, Fairbairn A, Perry EK (1990) Senile dementia of Lewy body type. A clinically and neuropathologically distinct form of Lewy body dementia in the elderly. J Neurol Sci 95:119–139
Pillon B, Michon A, Malapani C, Agid Y, Dubois B (1994) Are explicit memory disorders of progressive subranuclear palsy related to damage in striatofrontal circuits? Comparison with Alzheimer's, Parkinson's and Huntington's diseases. Neurology 44: 1264–1269
Pohl-Koppe A, Dahm C, Elgas M, et al (1992) The diagnostic significance of the polymerase chain reaction and isoelectric focusing in herpes simplex virus encephalitis. J Med Virol 36:147–154
Price JL, Davis PB, Morris JC, White DL (1992) The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. Neurobiol Aging 12:295–312
Prusiner SB (1993) Prion pathology. In: Prusiner SB, Collinge J, Powell J, Anderton B (eds) Prion diseases in humans and animals. Harwood, London
Prusiner SB (1993) Prion encephalopathies of animals and humans. Dev Biol

Stand 80:31–44
Puchhammer-Stöckl E, Heinz FX, Kundl M, et al (1993) Evaluation of the polymerase chain reaction for diagnosis of herpes simplex virus encephalitis. J Clin Microbiol 31:146–148
Reisberg B, Sclan SG, Franssen E, Deleon MJ, Kluger A, Torossian C, Shulman E, Steinberg G, Monteiro I, Mcrae T, Boksay I, Mackell J, Ferris SH (1993) Clinical stages of normal aging and Alzheimer's disease — the GDS staging system. Neurosci Res Commun 13:S51–S54
Reusche E (1991) Silver staining of senile plaques and neurofibrillary tangles in paraffine sections. Pathol Res Pract 187:1045–1049
Roberts GW, Nash M, Ince PG, Roystom MC, Gentleman SM (1993) On the origin of Alzheimer's disease: a hypothesis. Neuroreport 4:7–9
Roberts GW, Gentleman SM, Lynch A, Murray L, Landon M, Graham DI (1994) βamyloid protein deposition in the brain after severe head injury: implications for the pathogenesis of Alzheimer's disease. J Neurol Neurosurg Psychiatry 57:419–425
Roman GC, Tatechimi TK, Erkinjuntti T, et al (1993) Vascular dementia: diagnostic criteria for research studies. Report of the NINDS-AIREN International Workshop. Neurology 43:250–260
Samuel W, Terry RD, DeTeresa R, Butlers N, Masliah E (1994) Clinical correlates of cortical and nucleus basalis pathology in Alzheimer dementia. Arch Neurol 51:772–778
Scheff SW, Price DA (1993) Synapse loss in the temporal lobe in Alzheimer's disease. Ann Neurol 33:190–199
Schmidbauer M, Podreka I, Wimberger D, et al (1991) SPECT and MR imaging in herpes simplex encephalitis. J Comput Assist Tomogr 15:811–815
Selkoe DJ (1994) Alzheimer's disease: a central role for amylois. J Neuropathol Exp Neurol 53:438–447
Sparks DL, Liu H, Scheff SW, et al (1993) Temporal sequence of plaque formation in the cerebral cortex of nondemented individuals. J Neuropathol Exp Neurol 52:135–142
Sutton RC, Lipper MH, Brashear HR (1993) Limbic encephalitis occuring in association with Alzheimer's disease. J Neurol Neurosurg Psychiatry 56:808–811
Tatemichi TK, Desmond DW, Paik M, et al (1993) Clinical determination of dementia related to stroke. Ann Neurol 33:568–575
Tatemichi TK, Sacktor N, Mayeux R (1994) Dementia associated with cerebrovascular disease, other degenerative diseases, and metabolic disorders. In: Terry RD, Katzman R, Bikc KL (eds) Alzheimer disease. Raven Press, New York, pp 123–166
Terry RD, Masliah E, Salmon DP (1991) Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. Ann Neurol 30:572–580
Terry RD, Masliah E, Hansen LA (1994) Structural basis of the cognitive-alterations in Alzheimer disease. In: Terry RD, Katzman R, Bick KL (eds) Alzheimer disease. Raven Press, New York, pp 179–196
Tierney MC, Fischer H, Lewis AJ, et al (1988) The NINCDS-ADRDA Work Group criteria for the clinical diagnosis of probable Alzheimer's disease: clinicopathological study of 57 cases. Neurology 38:356–364
Tolosa E, Duvoisin R, Cruz-Sanchez FF (eds) (1994) Progressive supranuclear palsy: diagnosis, pathology, and therapy. J Neural Transm [Suppl 42]

Tomlinson BE (1992) Ageing and dementias. In: Adams JH, Duchen LW (eds) Greenfield's neuropathology, 5th ed. E Arnold, London, pp 1284–1410

Ulrich J, Spillantini MG, Goedert M, Dukas L, Stähelin HB (1992) Abundant neurofibrillary tangles without senile plaques in a subset of patients with senle dementia. Neurodegeneration 1:257–284

Van Hoesen GW, Hyman BT, Damasio AR (1991) Entorhinal cortex pathology in Alzheimer's disease. Hippocampus 1:1–8

Vermersch P, Delacourte A, Javoy-Agid F, Hauw JJ, Agid Y (1993) Dementia in Parkinson's disease, biochemical evidence for cortical involvement using the immunodetection of abnormal tau proteins. Ann Neurol 33:445–450

Vermersch P, Robitaille Y, Bernier L, et al (1994) Biochemical mapping of neurofibrillary degeneration in a case or progressive supranuclear palsy. Acta Neuropathol 87:572–577

Vollmer R, Ferbert A, Thron A (1993) Verlaufsbeobachtung bei paraneoplastischer limbischer Enzephalitis. Nervenarzt 64:659–662

Vonsattel JP, Myers RH, Stevens TJ, et al (1985) Neuropathological classification of Huntington's disease. J Neuropathol Exp Neurol 44:559–577

Wakabayashi K, Oyanagi K, Makifuchi T, Ikuta F, Hamma A, Hamma Y, Horikawa Y, Tokiguchi S (1994) Corticobasal degeneration: etiopathological significance of the cytoskeletal alterations. Acta Neuropathol 87:545–553

Watanabe R, Duchen LW (1993) Cerebral amyloid in human prion disease. Neuropathol Appl Neurobiol 19:253–260

West MJ (1993) Regionally specific loss of neurons in the aging human hippocampus. Neurobiol Aging 14:287–293

Wetterling T, Borgis KJ (1993) Vaskuläre Demenz. Ist eine klinische Unterteilung möglich und sinnvoll? Akt Neurol 20:40–48

Wolters ECH, Scheltens P (eds) (1993) Alzheimer's disease: back to the future. Vrije Universiteit, Amsterdam

Zhan SS, Beyreuter K, Schmitt HP (1993) Vascular dementia in Spatz-Lindenberg's disease (SLD): cortical synaptophysin immunohistochemistry as compared with dementia of Alzheimer's type and non-demented controls. Acta Neuropathol 86:259–264

Correspondence: Prof. Dr. K. Jellinger, Ludwig Boltzmann Institute of Clinical Neurobiology, Lainz Hospital, Wolkersbergenstrasse 1, A-1130 Vienna, Austria

Computed tomography and magnetic resonance imaging in the diagnosis of Alzheimer's disease

F.T. Aichner, S.R. Felber, and **G.G. Birbamer**

Universitätsklinik für Neurologie und Gemeinsame Einrichtung für Magnetresonanztomographie und Spektroskopie, Medizinische Fakultät, Leopold-Franzens-Universität, Innsbruck, Austria

Summary

Both cranial computerized tomography (CCT) and magnetic resonance tomography (MRT) are important tools in the clinical diagnosis of Alzheimer's disease. Volumetric CCT assessment and MRT analysis of brain, ventricular and intracavity volumes are mandatory for monitoring the progression of the disease, and have been highly standardized. Temporal lobe volume measurement by CCT and, in particular, by MRT, are useful for early diagnosis of AD and prospective studies of at risk populations. Both measurement of gray matter loss and white matter abnormalities give useful results, although the interpretation of white matter lesions in aged subjects may be difficult. MR spectroscopy, demonstrating significant chemical changes in aging brain and in AD subjects, suggests changes of phospholipid cell membranes in gray and white matter in AD and may be helpful in distinguishing AD from multiple infarctions. The specificity and sensitivity of recently developed MR spectroscopy tests remain to be established.

Introduction

The development and ageing of the human brain involve physiological and pathological changes that – although similar – qualitatively differ in quantity and topography. Ageing has variable effects on different brain regions and neuronal systems. While in the past only autopsy measurements could be performed, CT and MR-imaging are capable to demonstrate the brain development and the follow-up of normal ageing as well as degenerative brain disease (Jernigan et al., 1990; Schwartz et al., 1985). Previously, many speculative reports have indicated that the volume of human brain and distinct topographial features are related to intelligence, behaviour and personality of the

human being. The philosopher Immanuel Kant had a brain volume of about 1.500 ml and this was linked with his high intelligence. However, other geniuses had much smaller brain volumes, at least at the end of their lifes.

Dementia, a symptom complex, can be caused by more than 60 disorders. Alzheimer's disease (AD) accounts for 60%–80% of the cases and vascular disease for about 20%. Some patients have both disorders. The diagnostic work-up of dementia includes clinical assessment, neuropsychological and electrophysiological evaluation as well as morphological and metabolic studies. In the absence of a biological marker, the definitive diagnosis can only be made by biopsy and/or autopsy. While the accuracy of making the clinical diagnosis of AD is reasonably high – up to 90% – difficulties remain in differentiating early AD from the cognitive changes found in normal ageing (Aichner, 1993; Frackowiak, 1986; Hubbard et al., 1981; Marsden and Harrison, 1982; Ron et al., 1979; Tomlinson et al., 1970).

Methodology

For the CT and MR evaluation process of gray and white matter abnormalities, and brain volume loss, different methods are used: visual reading, rating scores, as well as planimetric and volumetric measurements (Aichner et al., 1988; Albert et al., 1984; Condon et al., 1988; Creasey et al., 1986). The morphological criteria of AD visualized on routine CT and MRI studies are showing temporal accentuated atrophy, hippocampal substance loss, ventricular enlargement, cortical atrophy and white matter foci. Visual reading is hampered by a very high intra- and interobserver variability. For nearly two decades, investigators using CT have attempted to quantify hemispheric cerebral atrophy in order to differentiate AD from cognitively normal elderly individuals. Semi-automated CT-based measurement techniques suffer from several inherent technical limitations and have been hampered by a lack of specifity. It is plausible that the most significant limitation in previous semi-automated CT-based attempts to quantify a global cerebral atrophy in patients with AD was that the areas of brain that are most severely involved (temporal lobe, hippocampal formation) were not included in the regions of interest. Several recent non-volumetric CT-studies in which the CSF spaces in the anteriotemporal region were perceptually evaluated have claimed impressive accuracy in separating patients with AD from elderly controls (Gado et al., 1983; George et al., 1990; Kido et al., 1989; Rezek et al., 1987; Sandor et al., 1988).

Previous attempts to apply one-dimensional linear measurements and subsequent attempts to apply two-dimensional planimetric measurements to CT images have been shown to be less accurate in measurement of CSF volumes. Wyper et al. (1979) reported a 20 to 30% error in ventricular volume measurements. George et al. (1990) described errors of 30–100% in measurements based on planimetry and CT. Penn et al. (1978) estimated an error of 16% using an interactive three-dimensional computer program to analyse CT images. MR-imaging eliminates the beam-hardening artifact and allows true multiplanar

imaging yielding more information for whole brain and regional volume analysis. MR provides higher decrease of contrast among CSF, brain, gray and white matter, adjustment of imaging parameters.

Volumetric assessment is mandatory for monitoring the progression of the disease and/or therapeutic interventions (Birbamer et al., 1993). 3D gradient echo sequences in T1 (FLASH) or relative T2 (PSIF) weightening allow imaging of the entire brain in nearly isotropic resolution ($0.8 \times 0.8 \times 1.2$ mm) (Felber et al., 1990). A high-speed computer work-station is necessary to synthesize this information into a multimodality, multivariable 3D-model by using tracing and thresholding techniques (Jack et al., 1989, 1990, 1992). A threshold used to discriminate structures in high contrast portions, whereas low contrast portions of the image are outlined by tracing. After selection of a proper threshold value, thresholding can be done automatically for all images. Tracing of low contrast regions, however, has to be done individually for each separate image and therefore constitutes the most time-consuming part of the segmentation procedure. After complete segmentation, the volume of the segmented structure is determed with a simple voxel-counting algorithm and the volume is attained by multiplying the total number of voxels by the volume of an individual voxel, which is given by the slice thickness, field of view and matrix size. Since this technique became feasible, accurate and time-sparing, an in-vivo brain data bank can be built up and used for the differential diagnosis of normal ageing and AD.

Foci of high signal in the cerebral white matter are common incidental findings on MR-images of the brain of control subjects or patients with a variety of diseases. Yetkin et al. (1992) found a high observer variability for high signal foci and the authors concluded that the number of foci of hyperintensity found in one study cannot be compared with a number in another unless a consensus has been reached between observers.

Comparing the applied methods of recently published MRI studies in AD it is obvious that there is a large amount of methodological variables, i.e. in regard to the magnetic field strength, pulse sequence, plane, slice thickness, study design, inclusion criteria, exclusion criteria and statistical analysis. Therefore, there is a strong need for a standardized method for future studies. On the other hand, MRI is still under development and has still a limited availability. A 3D-MRI software is not generally available and the measurement accuracy and reproducibility is not determined. One has to consider the high intra- and interobserver variation and the lack of validation studies. This lack can only be compensated by the initiation of MRI multicenter trials in AD.

Analysis of brain, ventricular and intracavity volumes with MR-imaging

Before the advance of neuroimaging, the determination of brain volume has been limited to post-mortem measurement by Archimedes' principle. Similarly the volume of the intracranial cavity has been determed at post-mortem by filling the skull with water or sand or by forming polyurethane casts. It has been realized since 1905 that the brain volume is of little value in itself because of the considerable

normal variations in head size between and within the sexes. The more appropriate parameter may be a measure of brain volume, normalised with respect to the intracranial cavity volume (Condon et al., 1988; Hubbard and Anderson, 1981).

In the last 15 years several results on the attempts of quantitation of brain, ventricular and intracavity volume using CT and MRI with different methods and computer programs have been published. The design of the studies are very heterogeneous, the sample size very small. The results can be summarized as inconclusive, even longitudinal studies using volumetric indices were not able to differentiate normal aging and the initial phase of Alzheimer's disease (Gado et al., 1982; George et al., 1983; Malko et al., 1991; Penn et al., 1978; Wyper et al., 1979). Recent studies, however, are very encouraging. Interobserver correlations are greater than 0.96 and the coefficients of variation in terms of absolute volume measurement are less that 7% and 2% respectively for CSF and brain (De Carli et al., 1992; Kohn et al., 1991; Tanna et al., 1991). Previous analyses of brain and CSF volumes with CT and MRI have to be interpreted carefully, the never computerised segmentation, 3D-MR-technique produces more accurate volumetric measurements of brain and CSF and yields significantly different group, that means separating healthy elderly control subjects from patients with Alzheimer dementia on the basis of absolute volume measurements obtained including total CSF, total ventricular and extraventricular CSF, total brain volumes and intracranial cavity volume.

Temporal lobe volume measurement

Selective atrophy of the anterior temporal lob, particular the hippocampal formation, is a pathological hallmark of AD, temporal lobe epilepsy and schizophrenia (Kesslak et al., 1991). MR may be able to detect atrophy in the temporal lobes earlier than CT, because the former can image the hippocampus and amygdala directly. CT temporal horn measurements greater than 3 mm occurred only in patients with AD, while measurements less than or equal to this occurred in both, Alzheimer patients and control subjects (Jack et al., 1992; Kido et al., 1989). For the evaluation of temporal lobe atrophy obliqued slices perpendicular to the hippocampus are mandatory in order to delineate parenchymal changes as well as tissue loss. We feel that while hippocampal volumetry is useful it will not provide an absolute diagnostic standard when applied prospectively to the population at risk for AD. Recently the interuncal distance was used for MR measurement for the hippocampal atrophy of AD. Thereby the interuncal distance appears to be a better measure of overall brain volume than of temporal lobe volume (Dahlbeck et al., 1991; Doraiswamy, 1993; Early et al., 1993).

Measuring of gray matter loss with MR-imaging

Ogata et al. (1973) measured the gray matter in 8 cadavers aged 53 to 83 years. The gray matter averaged 58.4% of the brain volume, a corrected value 46.7% is in agreement with the results of Rusinek et al. (1991).

To compare MR findings with the values reported in the neuropathologic literature, the post-mortem brain content of the gray matter has to be multiplied by 0.8. This correction is based on the assumption that the cranial cavity contains approximately 20% of CSF. De la Monte et al. (1989) measured the gray and white matter volume in 5 coronal brain sections of 17 patients with end-stage AD and 14 control subjects.

The difference in gray matter averaged 6% and ranged between 0 in a section at the frontal lobe and 13% in a section passing through the temporal lobe at the head of the caudate nucleus. Terry et al. (1981) reported a 10% reduction in the thickness of the cortical ribbon from 2.4 mm in healthy control subjects to 2.2 mm in patients with AD, further supporting the magnitude of gray matter loss in AD observed in the Rusinek MR study. The percentage of the gray matter in the brains of Alzheimer patients is significantly lower than in controls. The most significant reduction occurred in the temporal lobes and central region. The reduction of the frontal and occipital lobe was also statistically significant.

White matter abnormalities

Focal and confluent areas of periventricular hyperintensity are frequently seen on T2 weighted MR images of the brain in older subjects. The areas have been variously attributed to atrophic perivascular demyelination, to enlarged perivascular spaces, deep white matter infarction, ischemia without infarction and subependymal glial accumulation, fibrotic small blood vessels and Wallerian degeneration. Deep white matter infarction has previously been attributed to a hypertensive white matter vasculopathy, known as subcortical atherosclerotic encephalopathy (Braffman et al., 1988; Grafton et al., 1991; Kirkpatrick et al., 1987; Leiser et al., 1990; Marshall et al., 1988; Rosen et al., 1980).

Brun and Englund (1985) found symmetrical deep white matter changes at autopsy in more than 60% of patients with AD. White matter abnormalities occur in 15 to 30% of neurologically intact elderly persons, occur in 30 to 60% of patients with Alzheimer's disease, occur in most patients with vascular dementia and occur also in mood disorders (Bowen et al., 1990; Fazekas et al., 1987; Hendrie et al., 1989).

White matter changes in patients with dementia were recognised on CT and were termed leukoaraiosis. It was also seen on MRI and, in

general, they were often interpreted as being diagnostic for vascular dementia (Kobari et al., 1990). However, white matter abnormalities were increasingly reported in AD, too, but studies regarding the actual occurrence of white matter lesions in AD have yielded conflicting features of the dementia syndrome in AD is also still under debate (Erkinjuntti et al., 1987; Harrell et al., 1991; Leys et al., 1990; Liu et al., 1993; Lopez et al., 1992; Schmidt et al., 1992).

The MR signal behaviour of white matter lesions can be classified as hyperintense relative to brain parenchyma (PD, T2) indicating non-cystic infarction, gliosis or demyelination, and as isointense relative to CSF or fluids (T1, PD, T2) indicative of cystic infarction, brain cyst, prominent Virchow-Robin spaces, état criblé without surrounding parenchymal changes and ventricular diverticulum (Fazekas et al., 1987; Scheltens et al., 1993).

The differences in prevalence ranges can partly be explained by the use of different imaging modalities. Most of the MRI studies used the Fazekas rating scale to assess the pattern sensitivity of white matter hyperintensities (Fazekas et al., 1987, 1988). This scale provides only global information contrary to the CT method of scoring leukoaraiosis described by Rezek et al. (1987). Intraobserver reliability of the Fazekas method has been shown to be poor (Leys et al., 1990, 1991). Scheltens et al. (1992, 1993) designed a new rating scale in which periventricular and white matter signal hyperintensities as well as basal ganglia and infratentorial signal hyperintensities are rated separately in a semi-quantitative way. In their studies they confirmed the lower inter- and intraobserver agreements of the Fazekas scale. A new scale, also more elaborated provided good agreements with respect to the white matter basal ganglia and infratentorial signal hyperintensities. In rating periventricular hyperintensities alone, this scale yielded no advantage.

31P- and 1H-spectroscopy

Pettegrew et al. (1988) have reported alterations of phospholipid pathways detected by in-vitro 31P nuclear magnetic resonance spectroscopy, that might provide a quantitative method for distinguishing AD from other brain diseases. They found elevations of phosphomonoesters in the superior and middle frontal gyri and inferior parietal region of AD compared with non-diseased controls. Brains of patients with neurological diseases other than AD including those with multiple infarctions had elevations of phosphomonoesters at levels between AD and non-diseased controls. Further, levels of phosphomonoester in AD were inversely correlated with plaques suggesting the possibility that elevations of phosphomonoesters in AD were inversely correlated with plaques suggesting the possibility that elevations of phosphomonoesters might occur early in AD prior to plaque formation. The

same group used in-vivo 31 phosphor spectroscopy to study regional high energy phosphate and phospholipid metabolism in brains of patients with probable AD and multiple subcortical cerebral infarctions (Brown et al., 1989). The ischemic patients demonstrated elevations of the phosphocreatine, inorganic phosphate ratio, in both – the temporoparietal and frontal regions. Phosphomonoesters and the ratio of phosphomonoesters to phosphodiesters were elevated in the temporoparietal region of AD. Inorganic phosphor was also elevated in the frontal and temporoparietal regions of AD. The findings were interpreted as accurate in distinguishing AD from patients with multiple infarctions.

Bottomley et al. (1992) were unable to demonstrate significant abnormalities in metabolite concentrations or ratios in fully relaxed P31 NMR spectra from whole sections through the lateral ventricles in the brains of patients with probable AD. High-energy phosphate metabolism measured by these means does not appear to play a major role in the neuropathologic characteristics of the disease, except as a direct consequence of atrophy as quantified with MR imaging.

Proton MR-spectroscopy has been utilized to a much lesser extent in AD (Klunk et al., 1992). The preliminary 1H-NMR study of post-mortem AD brain suggests that the putative neuronal marker N-acetyl-aspartate can be used to follow the progression of neuronal loss in AD and perhaps other degenerative disorders. Perhaps more interesting is the finding that there appears to be a relative excess of glutamate per neuron that becomes larger as the disease progresses.

Miller et al. (1993) recently studied 11 elderly patients with mild or moderate dementia, assessed with standard neuropsychological tests. They described two abnormalities in the patients cerebral cortex: When compared with healthy subjects, the patients showed a 22% increase in myoinositole and an 11% decrease in residuals of N-acetyl-aspartate. The authors concluded that the combination of high myoinositole and low N-acetyl-aspartate at the examination with 1H-MR-spectroscopy may prove as an early diagnostic test for AD.

The specifity and sensitivity of such a test remain to be established.

References

Aichner F (1993) Clinical value of magnetic resonance imaging in the diagnosis of demential diseases. Psychiat Danub 5:177–187

Aichner F, Felber S, Willeit J (1988) Magnetic resonance imaging and computed tomography in diagnosis and assessment of dementia. In: Agnoli P, Cahn J, Lassen N, Mayeux R (eds) Senile dementia. Libbey, Paris, pp 213–217

Albert M, Naeser MA, Levine HL, Garvey AJ (1984) Ventricular size in patients with presenile dementia of Alzheimer type. Arch Neurol 41:1258–1263

Birbamer G, Felber S, Kampfl A, Aichner F, Gerstenbrand F, Benesch H (1993) Fast magnetic resonance imaging and 3D volumetric calculations in degen-

erative central nervous system diseases. In: Maurer K (ed) Imaging of the brain in psychiatry and related fields. Springer, Berlin Heidelberg New York Tokyo, pp 47–51

Bottomley PA, Cousins JP, Pendrey DL, Wagle WA, Hardy CJ, Eames FA, Mc Caffrey RJ, Thompson DA (1992) Alzheimer dementia: quantification of energy metabolism and mobile phosphoesters with P31 NMR spectroscopy. Radiology 183:695–699

Bowen BC, Barker WW, Loewenstein DA, Sheldon J, Duara R (1990) MR signal abnormalities in memory disorders and dementia. AJNR 11:283–290

Braffman BH, Zimmerman RA, Trojanowski J, Gonatas NK, Hickey WF, Schlaepfer WW, Brain MR (1988) Pathologic correlation with gross and histopathology. 2. Hyperintense white matter foci in the elderly. AJNR 9:629–636

Brown GG, Levine SR, Gorell JM, Pettegrew JW, Gdowski JW, Bueri JA, Helpern JA, Welch KMA (1989) In vivo 31 P NMR profiles of Alzheimer's disease and multiple subcortical infarct dementia. Neurology 39:1423–1427

Brun A, Englund E (1985) Alzheimer type dementia and white matter changes. Acta Neurol Scand 7:87–88

Condon B, Grant R, Hadly D, Lawrence A (1988) Brain and intracranial cavity volumes: in vivo determination by MRI. Acta Neurol Scand 78:387–393

Creasey H, Schwartz M, Frederickson H, Haxby JV, Rapoport SI (1986) Quantitative computed tomography in dementia of Alzheimer type. Neurology 36:1563–1568

Dahlbeck SW, Mc Cluney KW, Yeakley JW, Fenstermacher MJ, Bonmati C, Gage van Horn M (1991) The interuncal distance: a new MR measurement for the hippocampal atrophy of Alzheimer disease. AJNR 12:930–932

De Carli C, Maisog J, Murphy DGM, Teichberg D, Rapoport SI, Horwith B (1992) Method for quantification of brain, ventricular and subarachnoid CSF volumes from MR images. J Comput Assist Tomogr 16:274–284

De la Monte SM (1989) Quantification of cerebral atrophy in preclinical and end-stage Alzheimer's disease. Ann Neurol 25:450–459

Doraiswamy PM, Mc Donald WM, Patterson L, Husain MM, Figiel GS, Boyko OB, Krishnan KR (1993) Interuncal distance as a measure of hippocampal atrophy: normative data on axial MR imaging. AJNR 14:141–143

Early B, Escalowa PR, Boyko OB, Doraiswamy PM, Axelson DA, Patterson L, Mc Donald WM, Krishnan KRR (1993) Interuncal distance measurements in healthy volunteers and in patients with Alzheimer disease. AJNR 14:907–910

Erkinjuntti T, Ketonen L, Sulkava R, Sipponen J, Vuorialho M, Iivanainen M (1987) Do white matter changes on MRI and CT differentiate vascular dementia from Alzheimer's disease. J Neurol Neurosurg Psychiatry 50:37–42

Fazekas F, Niederkorn K, Schmidt R, Offenbacher H, Horner S, Bertha G, Lechner H (1988) White matter signal abnormalities in normal individuals. Correlation with carotid ultrasonography, cerebral blood flow measurements and cerebrovascular risk factors. Stroke 19:1285–1288

Fazekas F, Chawluk JB, Alavi B, Hurtig HI, Zimmerman RA (1987) MR signal abnormalities at 1.5 T in Alzheimer's disease dementia and normal aging. AJNR 149:351–356

Felber S, Birbamer G, Benesch H, Aichner F, Ehrike H (1990) Isotropic 3D-MR-imaging applied to degenerative disorders of the brain. In: Schneider GH, et al (eds) Digitale Bildgebung, interventionelle Radiologie, integrierte digitale Radiologie. Blackwell Ueberreuter, Cambridge, pp 282–285

Frackowiak RSJ (1986) Brain imaging in the assessment of the dementias. In: Poeck K, Freund HJ, Gänshirt H (eds) Neurology. Springer, Berlin Heidelberg New York Tokyo, pp 50–60

Gado M, Hughes CP, Danziger W, Chi D (1983) Aging, dementia and brain atrophy: a longitudinal computed tomographic study. AJNR 4:699–702

George AE, De Leon MJ, Stylopoulus LA, Miller J, Klinger A, Smith G, Miller DC (1990) CT diagnostic features of Alzheimer disease: importance of the chloroidal-hippocampal fissure complex. AJNR 11:101–107

Grafton ST, Sumi SM, Stimac GK, Alvord EC, Shaw Cerebrolysin, Nochlin D (1991) Comparison of post mortem magnetic resonance imaging and neuropathologic findings in the cerebral white matter. Arch Neurol 48:293–298

Harrell LE, Duvall E, Folks DG, Duke L, Bartolucci A, Conbow T, Callaway R, Kerns D (1991) The relationship of high-intensity signals on magnetic resonance images to cognitive and psychiatric state in Alzheimer's disease. Arch Neurol 48:1136–1140

Hendrie HC, Farlow MR, Austrom MG, Edwards MK, Williams MA (1989) Foci of increased T2 signal intensity on brain MR scans of healthy elderly subjects. AJNR 10:703–707

Hubbard BM, Anderson JM (1981) A quantitative study of cerebral atrophy in old age and senile dementia. J Neurol Sci 50:135–145

Jack CR Jr, Bentley MD, Twomey CR, Zinsmeister AR (1990) MR-imaging based volume measurements of the hippocampal formation and anterior temporal lobe: validation studies. Radiology 176:205–209

Jack CR Jr, Gehring DG, Sharbrough FW, Felmlee JO, Forbes G, Hench VS, Zinsmeister AR (1988) Temporal lobe volume measurement from MR images: accuracy and left-right asymmetry in normal persons. J Comput Assist Tomogr 12:21–29

Jack CR, Petersen RC, O'Brien PC, Tangalos EG (1992) MR-based hippocampal volumetry in the diagnosis of Alzheimer's disease. Neurology 42:183–188

Jernigan TL, Press GA, Hesselink JR (1990) Methods for measuring brain morphologic features on magnetic resonance images: validation and normal ageing. Arch Neurol 47:27–32

Kesslak JP, Nalcioglu O, Cotman CW (1991) Quantification of magnetic resonance scans for hippocampal and parahippocampal atrophy in Alzheimer's disease. Neurology 41:51–54

Kido DK, Caine ED, Lemay M, Ekham S, Booth H, Panzer R (1989) Temporal lobe atrophy in patients with Alzheimer disease: a CT study. AJNR 10:551–555

Kirkpatrick JB, Hayman LA (1987) White matter lesions in MR imaging of clinically healthy brains of elderly subjects: possible pathologic basis. Radiology 162:509–511

Klunk WE, Panchalingam K, Moossy J, Mc Clure RJ, Pettegrew JW (1992) N-acetyl-L-aspartate and other aminoacid metabolites in Alzheimer's disease brain: a preliminary proton nuclear magnetic resonance study. Neurology 42:1578–1585

Kobari M, Meyer JS, Ichijo M, Oravez WT (1990) Leukoaraiosis: correlation of MR and CT findings with blood flow, atrophy and cognition. AJNR 11:273–281

Kohn MI, Tanna NK, Herman GT, Resnick SM, Mozley PD, Gur RE, Alavi A, Zimmerman RA, Gur RC (1991) Analysis of brain and cerebrospinal fluid

volumes with MR imaging, part I. Methods, reliability and validation. Radiology 178:115–122
Leifer D, Buonanno FS, Richardson EP (1990) Clinicopathologic correlations of cranial magnetic resonance imaging of periventricular white matter. Neurology 40:911–918
Leys D, Soetaert G, Petit H, Fauquette A, Pruvo JP, Steiling M (1990) Periventricular and white matter magnetic resonance imaging hyperintensities do not differ between Alzheimer's disease and normal ageing. Arch Neurol 47:524–527
Lim KO, Pfefferbaum A (1989) Segmentation of MR brain images to cerebrospinal fluid spaces, white and gray matter. J Comput Assist Tomogr 13:588–593
Liu CK, Miller BL, Cummings JL, Mehringer CM, Goldberg MA, Howng SL, Benson DF (1989) A quantitative MRI study of vascular dementia. Neurology 42:138–143
Lopez OL, Becker JL, Rezek D, Wess J, Boller F, Reynolds III CF, Panisset M (1992) Neuropsychiatric correlates of cerebral white-matter radiolucencies in probable Alzheimer's disease. Arch Neurol 49:828–834
Malko JA, Hoffman JC Jr, Green RC (1991) MR measurement of intracranial CSF volume in 41 elderly normal volunteers. AJNR 12:371–374
Marsden CD, Harrison MJG (1972) Outcome of investigation of patients with presenile dementia. Br Med J 2:249–252
Marshall VG, Bradley WG, Marshall CE, Bhoopat T, Rhodes RH (1988) Deep white matter infarction: correlation of MR imaging and histopathologic findings. Radiology 167:517–522
Miller BL, Moats RA, Shonk T, Ernst T, Woolley S, Ross BD (1993) Alzheimer disease: depiction of increased cerebral myo-inositol with proton MR spectroscopy. Radiology 187:433–437
Mirsen TR, Lee DH, Wong CJ, Diaz F, Fox AJ, Hachinski VC, Merskey H (1991) Clinical correlates of white matter changes on magnetic resonance imaging scans of the brain. Arch Neurol 48:1015–1021
Ogata J, Feigin I (1973) The relative weight of the gray and white matter of the normal human brain. J Neuropathol Exp Neurol 32:585–588
Penn RD, Belanger MG, Yassnoff WA (1978) Ventricular volume in man computed from CT scans. Ann Neurol 3:316–223
Pettegrew JW, Panchalingam K, Moossy J, Martinez J, Rao G, Boller F (1988) Correlation of phosphorous-31 magnetic resonance spectroscopy and morphologic findings in Alzheimer's disease. Arch Neurol 45:1093–1096
Rezek DL, Morris J, Fulling KH, Gado MH (1987) Periventricular white matter lucencies in senile dementia of Alzheimer type and in normal ageing. Neurology 37:1365–1368
Ron MA, Toone BK, Garralda ME, Lishman WA (1979) Diagnostic accuracy in presenile dementia. Br J Psychiatry 136:161–168
Rosen WG, Terry RD, Fuld PA, Katzman R, Peck A (1980) Pathological verification of ischemia score in differentiation of dementia. Ann Neurol 7:486–488
Rusinek H, De Leon JM, George AE, Stylopoulos LA, Chandra R, Smith G, Rand T, Maurino M, Kowalski H (1991) Alzheimer disease: measuring loss of cerebral gray matter with MR imaging. Radiology 178:109–114
Sandor T, Albert M, Stafford J, Harpley S (1988) Use of computerized CT analysis to discriminate between Alzheimer patients and normal control subjects. AJNR 9:1181–1187

Scheltens P, Barkhof F, Valk J, Alsgra PR, Gerritsen van der Hoop R, Nauta J, Wolters EC (1992) White matter lesions on magnetic resonance imaging in clinically diagnosed Alzheimer's disease. Evidence for heterogeneity. Brain 115:735–748

Scheltens P, Barkhof F, Leys D, Pruvo JP, Nauth J, Vermersch P, Steinling M, Valk J (1993) A semiquantitative rating scale for the assessment of signal hyperintensities on magnetic resonance imaging. J Neurol Sci 114:7–12

Schmidt R, Fazekas F, Kleinert G, Offenbacher H, Gindl K, Payer F, Freidl W, Niederkorn K, Lechner H (1992) Magnetic resonance imaging signal hyperintensities in deep and subcortical white matter: a comparative study between stroke patients and normal volunteers. Arch Neurol 49:825–827

Schmidt R (1992) Comparison of magnetic resonance imaging in Alzheimer's disease, vascular dementia and normal aging. Eur Neurol 32/3:164–169

Schwartz M, Creasey H, Grady CL, Deleo JM, Frederickson HA, Cutler NR, Rapoport SI (1985) Computertomographic analysis of brain morphometrics in 30 healthy men, aged 21 to 81 years. Ann Neurol 17:146–157

Tanna NK, Kohn MI, Horwich DN, Jolles PR, Zimmerman RA, Alves WM, Alavi A (1991) Analysis of brain and cerebrospinal fluid volumes with MR imaging: impact on PET data correction for atrophy, part II. Aging and Alzheimer dementia. Radiology 178:123–130

Terry RD, Peck A, De Theresa R, Schechter R, Horoupian DS (1981) Some morphometric aspects of the brain in senile dementia of the Alzheimer type. Ann Neurol 10:184–192

Tomlinson BE, Blessed G, Roth M (1970) Observations on the brains of demented old people. J Neurol Sci 11:205–242

Wippold II FJ, Gado MH, Morris JC, Duchek JM, Grant EA (1991) Senile dementia and healthy ageing: a longitudinal study. Radiology 179:215–219

Wyper DJ, Pickard JD, Matheson M (1979) Accuracy of ventricular volume estimation. J Neurol Neurosurg Psychiatry 42:345–350

Yetkin FZ, Hanghton VM, Fischer ME, Papke RA, Daniels DL, Mark LP, Hendrix LE, Asleson RJ, Johansen J (1992) High signal foci on MR images of the brain: observer variability in their quantification. AJR 159:185–188

Correspondence: Univ.-Prof. Dr. F. Aichner, Universitätsklinik für Neurologie und Gemeinsame Einrichtung für MR-Tomographie, Anichstrasse 35, A-6020 Innsbruck, Austria

Diagnostic imaging techniques with special reference to PET

W.-D. Heiss

Max-Planck-Institut für neurologische Forschung und Neurologische Universitätsklink, Köln, Federal Republic of Germany

Summary

The late occurrence of gross morphologic changes in Alzheimer's disease (AD) and the broad overlap of these alterations with those in normal age-matched controls preclude the use of CT and MRI for differential diagnosis of dementia syndromes. Because progressive cell loss and reduced cell and synaptic activity lead to a reduction in metabolism and blood flow, functional imaging techniques visualizing these variables can be helpful in detecting early alterations in AD. Positron emission tomography (PET) is currently the only technology affording three-dimensional measurement of the brain's energy metabolism which is closely coupled to brain function. Studies of glucose metabolism by PET of (18F)-2-fluoro-2-deoxy-D-glucose are therefore widely applied to show the contribution of various brain structures in the performance of a variety of tasks or their participation in functional deficits associated with various diseases. Although glucose metabolism decreases slightly with age to a regionally different degree, most types of dementia show severe changes in glucose metabolism. Alzheimer's disease (AD) is characterized by metabolic disturbances most prominent in the parietotemporal association cortex and later in the frontal lobe, whereas primary cortical areas, basal ganglia, thalamus, brainstem, and cerebellum are not affected. It is this typical pattern that distinguishes AD from other dementia syndromes. A ratio calculated from the metabolic rates of glucose of "affected" and "nonaffected" brain regions was able to separate patients with AD from age-matched controls and permitted the discrimination of patients with cognitive impairment of other origin in 85%. The discriminative power can be further improved by activation studies. A continuous visual recognition task increased the metabolic rate in normal subjects by 21% and in patients with AD by 6% on average, with significant regional differences. During activation the significant relation between severity of disease and temporoparietal metabolic rate became even stronger. In the assessment of effects of treatment on disturbed metabolism, PET studies demonstrated an equalization of metabolic heterogeneities in patients responding to a muscarinergic cholinergic agonist, whereas general increases in glucose

utilization were observed with piracetam, pyritinol, and phosphatidylserine. The therapeutic relevance of such metabolic effects, however, must be proved in controlled clinical trials. Preliminary results in 4 groups of AD receiving either social support, or cognitive training alone, or cognitive training combined with medical treatment for 6 months suggest that neuropsychological performance and activated glucose metabolism can be improved by therapeutic interventions targeted to special symptoms.

Introduction

Current research criteria for the diagnosis of Alzheimer's disease (AD) (McKhann et al., 1984) are based on clinically apparent progressive dementia occurring in middle or late life, the documentation of cognitive and memory deficits by appropriate neuropsychological testing and the exclusion of other diseases by various laboratory tests responsible for the cognitive decline or the personality changes. Since these criteria permit only the conclusion of "probable AD" and, therefore, leave some uncertainty on the exactness of the diagnosis, there is still a need for more accurate diagnostic markers especially when comparable groups of patients should be included in clinical and therapeutic research studies of the disease. As indicated in Table 1, the accurate histologic and biochemic diagnosis of AD can only be made after brain biopsy or autopsy. CT and MRI show brain atrophy at progressed stages of the disease, while EEG and single photon emission tomography (SPECT) provide some information on function and slected physiologic variables, especially cerebral blood. The late occurrence of gross morphologic changes in AD (Fig. 1) and the broad overlap of these alterations with those in normal, age-matched controls preclude the use of computed tomography (CT) and magnetic resonance imaging (MRI) for early differential diagnosis. Since progressive cell loss and reduced cell and synaptic activity lead to a reduction of metabolism and blood flow, functional imaging techniques visualizing these variables can be helpful in detecting early alterations in AD.

Table 1. Diagnostic value of various clinical methods in the assessment of pathologic changes in dementia

	Morphology	Chemistry	Physiology
EEG	−	−	+
CT	++	−	−
MRI	+++	+	+
PET	−	+	+++
SPECT	−	−	++
Biopsy	+++	++	+

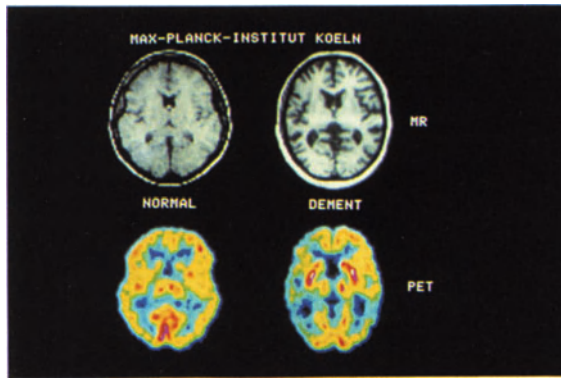

Fig. 1

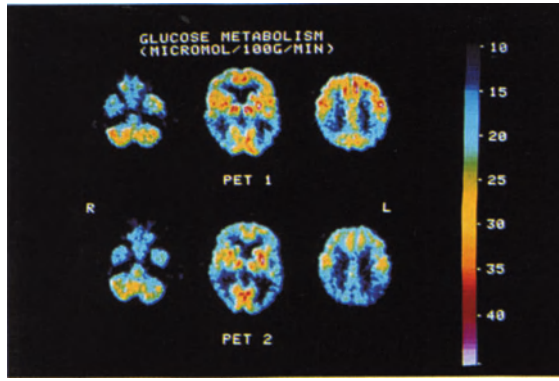

Fig. 2

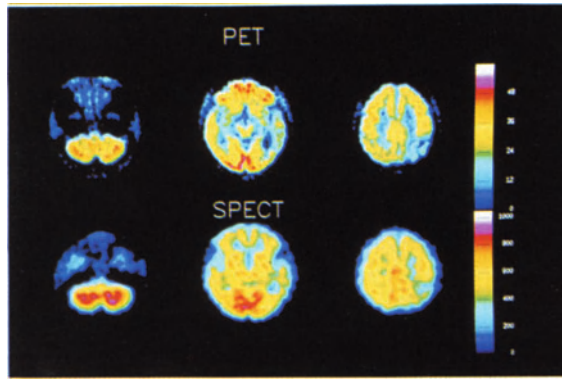

Fig. 4

Fig. 1. Matched MRI- and FDG-PET images of a patient suffering from Alzheimer dementia and an age-matched healthy volunteer demonstrating typical metabolic decreases in parieto-temporal and frontal association cortex which cannot be seen on MRI

Fig. 2. Progress of metabolic disturbance with duration of disease in a patient suffering from mild to moderate AD. Interval between studies was 1 year

Fig. 4. Comparison of matched FDG-PET and HM-PAO SPECT images (cerebellar, temporo-basal, and centrum semiovale level) of a patient with AD showing similar pattern and good correlation of regional values (r = 0.69)

Positron emission tomography (PET) is currently the only technology affording three-dimensional measurement of the brain's energy metabolism which is closely coupled to brain function, oxygen metabolism and blood flow. State-of-the-art PET scanners are equipped with up to 24 detector rings simultaneously scanning up to 47 slices 5–10 mm in thickness, at an in-slice spatial resolution of 5–10 mm. Gray- or pseudocolor-coded tomographic images of the radioactivity distribution are reconstructed by dedicated computers from the many projected coincidence counts, using CT-like algorithms and reliable scatter and attenuation correction. State of the art equipment permits reformation of brain images in any orientation and show a good correpondence to morphologic images, e.g., MRI, corregistered in the same individuals (Fig. 1). Details on the physical properties of the applied PET equipment, the data collection and analysis procedures may be taken from the original publications (Heiss et al., 1984; Wienhard et al., 1992).

Since radioactivity tomograms only represent local tracer concentrations that do not have a direct meaning in a physiological sense, biomathematical models are needed to describe the compartmental kinetics of the radiotracers applied so that the data can be transformed into images of truly biological function. Several models require the collection of data by sequential PET scanning for dynamic analysis.

The cerebral energy supply necessary for any function of brain tissue depends almost exclusively on oxydative glucose metabolism, and therefore the most widely used approach to the imaging of memory functions and their disturbances involves the determination of the cerebral metabolic rate of glucose (CMR_{glu}). With few exceptions, 2 (^{18}F)fluoro-2-deoxy-D-glucose (FDG) is chosen for this purpose, and CMR_{glu} is quantified according to the three compartment operational equation developed for autoradiography by Sokoloff et al. (1977), using modifications for PET data in humans (Phelps et al., 1979; Reivich et al., 1979; Wienhard et al., 1985).

PET images are usually analyzed first by visual interpretation by an experienced observer. Registration to corresponding CT or MRI scans is required for point-to-point matching of function and structure (Evans et al., 1988; Friston et al., 1990; Pietrzyk et al., 1990). For statistical assessment, however, comprehensive brain mapping into meaningful regions of interest or subtraction procedures of individual or pooled data, are well established. All these approaches entail averaging and/or partial volume errors, but they improve data comparability. Significance probability mapping can help explore effects in single cases, while repeated-measures analysis of variance handles multiregional multigroup hypotheses most efficiently (Pawlik, 1988), and exploratory pixel-by-bixel t-test procedures yield distinct regional hypotheses on the changes of physiologic variables due to external stimulation (Friston et al., 1990).

Primary degenerative dementias

In the degenerative dementias, especially in AD, impairment of memory and cognition corresponds with metabolic abnormalities long before morphological changes are detectable by neuroimaging tools. A typical pattern of decreased metabolism (Fig. 1), most prominent in the parieto-temporal association cortex and later in the frontal cortex, without affection of primary cortical areas, basal ganglia, thalamus and cerebellum, were consistently reported by many authors (Benson et al., 1983; DeLeon et al., 1983; Duara et al., 1986; Friedland et al., 1983; Haxby et al., 1986; Kuhl et al., 1983; Szelies et al., 1986). The characteristically decreased regional CMR_{glu} in temporo-parietal and frontal association areas further deteriorated with progression of the disease (Fig. 2) and was correlated to severity of the dementia. This typical pattern can help to distinguish AD from dementias of other causes (Heiss et al., 1989), and a metabolic ratio $^{(R)}$ of typically affected to typically unaffected regions achieved high sensitivity and specificity in classifying patients as AD or non-AD (Herholz et al., 1990). In early AD presenting only with memory impairment or mild dementia, the slight functional abnormalities may readily escape detection by PET unless such discriminating metabolic ratios are used (Friedland et al., 1983; Haxby et al., 1986; Kuhl et al., 1983). Of all the tested regional values, the metabolic rate of glucose in temporo-parietal association cortex showed the closest correlation with the severity of clinical symptoms (Fig. 3), while CMR_{glu} in sensorimotor cortex did not change with the degree of dementia (Kessler et al., 1991).

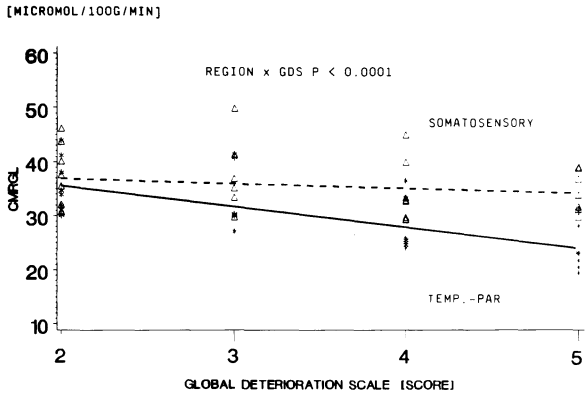

Fig. 3. Diagram of relationship between severity of dementia and regional metabolic rate of glucose: there is a significant correlation of score in global deterioration scale (GDS) to glucose metabolism in the temporo-parietal association area, but not to CMR_{glu} in sensorimotoric cortex

The individually dominating neuropsychological deficit obviously shows some relation to the extent of regional metabolic disturbance within the typical AD pattern: While a mainly amnestic disorder was related to bilateral temporal metabolic decreases, verbal incompetency correlated with the metabolic decrease in left temporo-parietal cortex, changes in visuoconstructive performance and apraxias were associated with right parietal lobe dysfunction (Chase et al., 1984; Foster et al., 1983, 1986; Riege et al., 1987).

The accuracy of the metabolic ratio (R) was also tested in an independent group of 56 patients. The mean R in those without objective disease was 1.07 ± 0.05 (n = 8), in those with Binswanger's disease or multi-infarct syndrome 1.10 ± 0.06 (n = 7), in other non-AD patients 1.02 ± 0.07 (n = 15), and in patients with probable AD 0.86 ± 0.11. Some overlap was noted between patients with AD and normal subjects or non-AD patients, respectively, in the metabolic ratio range of 0.90 − 1.03. Overlap was in part caused by the somewhat lesser severity of AD in the present sample than in the previous patient group used to establish the diagnostic criteria, and by seven "non-AD" patients with Parkinson's disease, hypoxic or hypoglycemic brain damage, amyotrophic lateral sclerosis, chronic encephalitis, and Wernicke's encephalopathy. Interestingly, no overlap occurred between patients with AD and those with cerebrovascular disease.

A study of 14 presenile (age 52–61 years) and 24 senile (age 66–81 years) demented patients (Mielke et al., 1991) indicated less contrast in $rCMR_{glu}$ between the areas typically affected and those not affected by AD in the senile cases. As a consequence, the ratio in AD approached 1 with age, making the distinction from normal subjects older in age (control group, age 51–77 years) more difficult. However, a significant relation existed between severity of dementia and absolute $rCMR_{glu}$ in the temporo-parietel association cortex that was independent of age. These findings support the view that presenile and senile dementia of the Alzheimer type is not a homogeneous entity and demonstrate that the severity of cognitive impairment is best correlated to absolute $rCMR_{glu}$ irrespective of the subgroup of AD.

Principally, similar changes of regional brain perfusion in AD can be observed by HM-PAO SPECT (Fig. 4). In a comparative study (Table 2) on 20 patients with AD, 12 patients with vascular dementia and 13 age-matched controls, however, the discriminative power of HM-PAO SPECT was found to be not as high as that of FDG-PET (Mielke et al., in preparation). In some cases, especially those with mild and early dementia, the typical changes could not be clearly detected in the SPECT images (Fig. 5). Therefore, specificity and sensitivity of SPECT was below that of PET, which is probably partly due to technical differences between these two methods. Differences of the measured variable, however, must be additionally taken into consideration: The results might indicate that metabolic disturbances are more sensitive to

Table 2. Comparison of ratio between affected and non-affected regions assessed by FDG-PET and HM-PAO SPECT in Alzheimer's disease (AD), vascular dementia (VD), and age-matched controls

	AD (n = 20)	VD (n = 12)	Controls (n = 13)
Ratio PET	0.89 ± 0.06 (0.80–0.99)***	1.0 ± 0.06 (0.92–1.1)	0.97 ± 0.02 (0.94–1.02)
Ratio SPECT	0.87 ± 0.05 (0.76–0.96)**	0.92 ± 0.08 (0.81–1.1)	0.94 ± 0.06 (0.85–1.0)

** $p < 0.01$ (AD − controls); *** $p < 0.0001$ (AD − controls)

functional impairment due to synaptic changes, while flow alterations occur later when metabolism is more affected and morphologic changes, especially neuronal loss, are more severe.

Because AD affects mainly and primarily parieto-temporo-occipital areas involved in processing visual information, a continuous visual recognition test adapted to the individual's performance was developed to activate those regions specifically and to quantify the capacity to respond to special demands. CMR_{glu} was measured at rest and during stimulation in 21 patients with probable AD (age 65.4 ± 7.21 years) and in 9 age-matched healthy control subjects (62.5 − 7.4 years) (Kessler et al., 1991). The patients with AD were mildly to moderately demented and scored between 3 and 6 on the GDS scale (Reisberg et al., 1982). The continuous visual recognition task increased the global metabolic rate in the controls by 6.6 ± 5.5 μmol/100g/min (21 ± 18% of resting values of 31.7 ± 4.34 μmol/100g/min); in patients with AD (Fig. 6) the metabolic change during activation (1.5 ± 3.00 μmol/100g/min = 5.7 ± 11.1% of resting value 29.7 ± 4.69 μmol/100 g/min) was significantly smaller ($p = 0.023$). Due to the test design the activation of $rCMR_{glu}$ was most prominent in the visual cortex and the temporo-parietal association areas, but the complex task additionally involved widespread brain structures, increasing $rCMR_{glu}$ differently (Fig. 6). Neither at rest nor during stimulation did the $rCMR_{glu}$ of the structures usually less involved in AD, such as the sensorimotor cortex, show a relation to the severity of dementia as assessed by the global deterioration scale (GDS). A significant correlation between $rCMR_{glu}$ in areas usually affected by AD pathology, such as the temporoparietal cortex, and GDS scores was found ($rho = -0.057$, $p = 0.001$), and it became stronger during metabolic activation by the visual recognition task ($rho = -0.60$, $p = 0.001$). A similar relation was observed between the severity of dementia assessed by Mini-Mental State Examination and synaptic density in brain biopsies (DeKosky and Scheff, 1990). From these results it can be concluded that the metabolic rate at rest estimates the extent on morphologic damage, whereas PET studies during activation indicate the brain's reserve capacity to respond to functional tasks. Because metabolism during activation in AD patients is more severely impaired

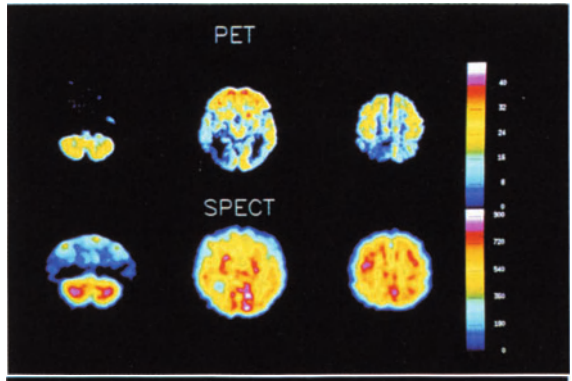

Fig. 5

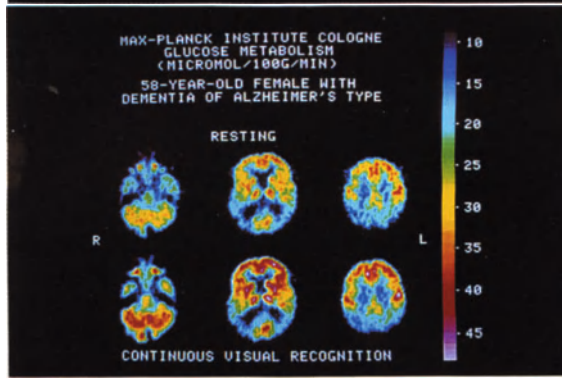

Fig. 6

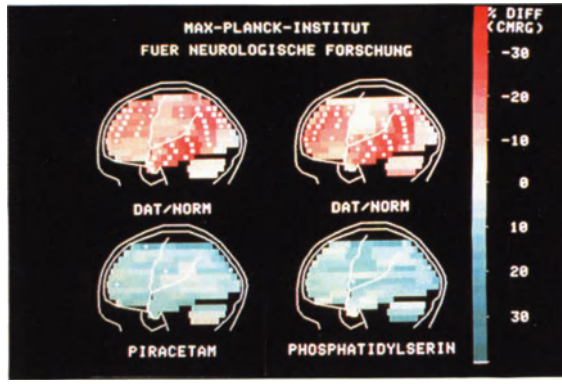

Fig. 8

Fig. 5. Comparison of matched FDG-PET and HM-PAO SPECT images (cerebellar, temporal, centrum semioval level) in a patient with moderate AD: the pathologic pattern visible in PET is not clear in SPECT, correlation of regional values is poor (r = 0.21)

Fig. 6. Response of glucose metabolism (upper row at rest) to activation by continuous visual recognition task (low row, slices through cerebellum, basal ganglia and centrum semiovale) in a patient with moderate AD. The typical affected regions show decreased response to stimulation

Fig. 8. Typical cortical pattern of metabolic disturbances in two groups of AD (upper row) in comparison to age-matched controls, and changes induced by piracetam (left) and phosphatidylserine (right). Both drugs increase metabolic rate diffusely, with signifi cant changes in several regions (+)

then that at rest, PET studies during functional tests could help in the selection of patients with a potential to benefit from therapeutic intervention.

In the second, but much rarer form of primary degenerative dementia – Pick's disease – the first and most marked metabolic changes – in analogy to the primary localization of pathological changes – are seen in the frontal and temporal lobe (Szelies and Karenberg, 1986). This distinctly different pattern of damage allows Pick's disease to be differentiated from AD; in moderate cases it is often not possible to make this distinction on the basis of clinical findings alone. The typical reduction of metabolism in the (frequently asymmetrically affected) atrophic frontal lobes and lower temporal lobes and in the much less changed parietal lobes as well as the basal ganglia and the thalamus is correlated with the degree of gliosis and cell depletion (Kamo et al., 1987).

The pattern of metabolic disturbance is also characteristic in Huntington's chorea which, in addition to the extrapyramidal-hyperkinetic syndrome, is always accompanied by dementia disorders. The glucose turnover rate in the neostriatum is already significantly reduced in the early stages of this disease and as the severity and duration of the disease increases, metabolism is seen to be reduced in the caudate nucleus and putamen, and later (according to the degree of severity of dementia) also the cerebral cortex (Kuhl et al., 1984). Since the metabolic disturbances precede the clinical manifestations of the disease, PET studies may possibly help to identify persons at risk in chorea families, and these studies as well as genetic investigations can be used to establish a prognosis for the subsequent appearance of the disease (Hayden et al., 1987; Mazziotta et al., 1987).

In Parkinson's disease, a degeneration of the dopaminergic nigrostriatal system, glucose metabolism is usually not altered, in contrast to the reduction of the dopaminergic endings in the basal ganglia demonstrated by means of PET of 18F-dopa (Nahmias et al., 1985). Only on development of a dementia, a frequent concomitant of Parkinson's disease, are the metabolic changes typical of AD also in evidence (Kuhl et al., 1985).

Vascular dementias

Focal cerebral lesions caused by blood flow disturbances can induce dementia syndromes through two mechanisms in particular: multiple lesions in mostly neurologically silent, frequently subcortical regions impair cerebral function in the form of dementia (multi-infarct dementia) when they exceed a total volume that cannot be precisely defined (80–150 cm^3). In rare cases, relatively small infarcts of critical localization can cause dementia syndrome in addition to the focally

dependent neurological symptoms. Chronic inadequate blood flow in the cerebral tissue which leads to a persisting hypofunction and thus to a disturbance of intellectual function which cannot be precisely localized, is likely to be present only in exceptional cases or to be of temporary duration following transient blood flow disturbances. Such deficient perfusion syndromes probably are only very rarely a cause of dementia since in the usual forms no corresponding disproportions between blood flow and oxygen consumption or glucose metabolism could be demonstrated (Frackowiak et al., 1981; Gibbs et al., 1986).

Multi-infarct dementias (MID) together with the AD-MID mixed forms account for about 30% of all dementia syndromes. A clinical differentiation on the basis of rating scales (Hachinski et al., 1975) is often difficult, and diagnostic classification is often easier on the basis of morphological lesions demonstrated by CT or MRI. In MID patients, PET can clearly differentiate mostly multilocular metabolic reductions from the pattern typical of AD (Kuhl et al., 1983). Detection of ischemic lesions in the medullary layer in MID and Binswanger's disease can be performed with great sensitivity by means of T2-weighted MRI (Heiss et al., 1986; Alavi et al., 1987), and the regions of reduced metabolism then correspond to the superjacent deafferentated cortical areas. Small localized infarcts in strategically important regions, for example unilaterally in the anterior nucleus of the thalamus or bilaterally in the median thalamus lead to permanent cognitive and amnestic losses.

Korsakoff's syndrome is another example of a relatively selective memory disturbance. Here the memory disturbance particularly affects events in the recent past (short-term memory) with attempts being made to compensate these deficits by confabulation. The most frequent cause is thiamine deficiency in chronic alcohol abuse, although several other diseases may also cause similar memory impairments. CT scans of patients with alcohol-related Korsakoff's syndrome showed diffuse moderate cerebral atrophy, while metabolism was diffusely moderately reduced and was disturbed especially bilaterally in the hippocampus, hypothalmus and thalamus.

Evaluation of drug effects in dementia

As long as the molecular biologic changes in AD cannot be influenced by any form of intervention, symptom-oriented therapy aims at improving loss of memory, the hallmark of the disease, and other symptoms relevant to daily life. For that purpose, attempts to correct the loss of acetylcholine, other neurotransmitters, or peptides, and enhancement of the metabolism of remaining neurons are now being employed. As impairment of memory and other cognitive disturbances can be related to decreased glucose metabolism in brain regions predominantly affected by AD, improvement in glucose utilization may

prevent further cognitive decline. Therefore, studies of $rCMR_{glu}$ by PET may be of value in the preclinical evaluation of drug therapy in this degenerative disorder.

The use of PET to study objectively the effects of drugs is still rare and has so far been limited to small groups of patients. Glucose metabolism was monitored for 6–12 weeks in eight patients with AD of differing severity who were undergoing therapy with the muscarinic choline agonist RS 86 (Sandoz, 2.5–3 mg/day) (Szelies et al., 1986). Over this period the global metabolic rate decreased under therapy, but compensation in the heterogeneous metabolic pattern typical of AD was noted with a particular reduction in the slightly elevated values (sensorimotor and visual cortex) measured before starting treatment, and there was only a slight influence on the typically lowered parieto-occipital to temporal values. This effect was especially pronounced in patients whose condition clinically stabilized on this therapy and who showed improved performance in several functions; this group, who profited from therapy, originally showed regional glucose metabolic rates that diverged relatively little from the norm and were also those whose AD was less severe. This study, therefore, shows the importance of initiating therapy at an early stage before severe cell destruction and suggests that a metabolic decoupling between different brain regions contributes to the specific symptoms.

Another study (Heiss et al., 1988) examined whether piracetam, which improves memory performance when administered in combination with precursors of acetylcholine (Ferris et al., 1982; Smith et al., 1984), has metabolic effects in AD. Of 16 patients with dementia

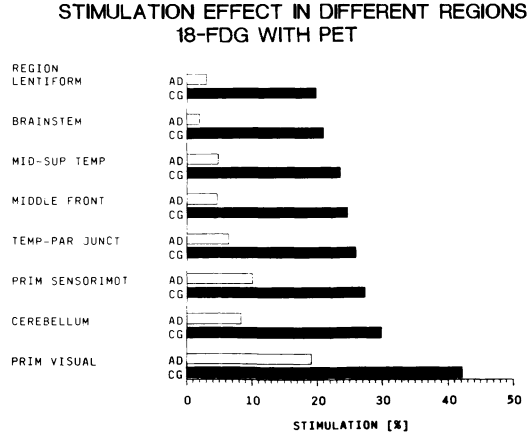

Fig. 7. Mean response of various brain regions to continuous visual recognition task in AD and healthy controls. The response to the task is significantly reduced, especially in the association areas

syndrome nine fulfilled the criteria for AD. The remaining seven were graded as MID or unclassifiable and used as a control group. Between the PET investigations, all patients received 6 g piracetam twice daily (Nootrop^R, UCB Chemie) for 14 days as a short infusion. The groups differed significantly from each other and from a control group of similar age with respect to regional rates of glucose metabolism, the reductions being particularly pronounced in the parietotemporo-occipital regions for the AD groups (Fig. 8). With piracetam treatment the glucose metabolism values in the AD group increased in the frontal, central, parieto-occipital, visual, auditory and cingulate crotex, basal ganglia, and thalamus, whereas no significant changes were detected in the non-AD group. The differences in the effects of treatment between AD and non-AD groups were statistically significant (ANOVA $p < 0.02$ for interactions between regions, treatment, and group); on the basis of the ANOVA the increase in the individual regions was checked by paired t test. The results were supported by improvements in five patients with AD during the short-therapy phase with respect to their clinical deficits and their performance in tests. Similar results were obtained in eight patients with AD under treatment with phosphatidyl serine (FIDIA, 500 mg/day for 3 weeks), which is suggested to have an effect on membrane structure and cell function (Fig. 8). In these patients $rCMR_{glu}$ increased by 14.8% during treatment (repeated measures ANOVA $p < 0.01$), with the most significant effects in basal ganglia (20.3%) and the visual cortex (19.3%) (Klinkhammer et al., 1990).

Preliminary results with pyritinol (Encephabol^R Merck, 600 mg daily for 10 weeks) showed some effect on $rCMR_{glu}$ in affected as well as in unaffected brain regions. These results justified a larger controlled long-term trial, in which 80 patients with probable AD were randomly assigned to 4 groups each receiving either social support, or cognitive training alone or cognitive training in combination with pyritinol (2×600 mg/d) or phosphatidyl serine (4×100 mg/d). A preliminary analysis of the results indicated improved neuropsychological performance and increased metabolic response to the activation task in the groups receiving specific medical treatment in combination with cognitive training (Heiss et al., 1993). This longitudinal study demonstrates that synaptic and neuronal function might be improved and progress of the disease might be mitigated in AD by specific treatment as long as there are neuronal networks preserved which are able to respond to tasks and thereby form a target for therapeutic intervention. However, large scale controlled clinical trials will be needed to support these data and to justify widespread clinical use.

References

Alavi A, Fazekas F, Chawluk J, Zimmerman R (1987) Magnetic resonance imaging of the brain in normal aging and dementia. In: Meyer JS, Lechner

H, Reivich M, Ott EO (eds) Cerebral vascular disease 6. Excerpta Medica, Amsterdam New York Oxford, pp 191–195

Benson DF, Kuhl DE, Hawkins RA, Phelps ME, Cummings JL, Tsai SY (1983) The fluorodeoxyglucose 18 F scan in Alzheimer's disease and multiinfarct dementia. Arch Neurol 40:711–714

Chase TN, Fedio P, Foster NL, Brooks R, Di Chiro G, Mansi L (1984) Wechsler adult intelligence scale performance. Cortical localization by fluorodeoxyglucose F-18 positron emission tomography. Arch Neurol 41:1244–1247

DeKosky ST, Scheff SW (1990) Synapse loss in frontal cortex biopsies in Alzheimer's disease: correlation with cognitive severity. Ann Neurol 27:457–464

DeLeon MJ, Ferris SH, George AE, Reisberg B, Christman DR, Kricheff II, Wolf AP (1983) Computed tomography and positron emission transaxial tomography evaluations of normal aging and Alzheimer's disease. J Cereb Blood Flow Metab 3:391–394

Duara R, Grady C, Haxby J, Sundaram M, Cutler NR, Heston L, Moore A, Schlageter N, Larson S, Rapoport SI (1986) Positron emission tomography in Alzheimer's disease. Neurology 36:879–887

Evans AC, Beil C, Marrett S, Thompson GJ, Hakim A (1988) Anatomical-functional correlation using an adjustable MRI-based region of interest atlas with positron emission tomography. J Cereb Blood Flow Metab 8:513–530

Ferris SH, Reisberg B, Crook T, Friedman E, Schneck K, Mir P, Sherman KA, Corwin J, Gershon S, Bartus RT (1982) Pharmacologic treatment of senile dementia: choline, L-dopa, piracetam, and choline plus piracetam. Aging 19:475–481

Foster NL, Chase TN, Fedio P, Patronas NJ, Brooks RA, DiChiro G (1983) Alzheimer's disease: focal cortical changes shown by positron emission tomography. Neurology 33:961–965

Foster NL, Chase TN, Patronas NJ, Gillespie MM, Fedio P (1986) Cerebral mapping of apraxia in Alzheimer's disease by positron emission tomography. Ann Neurol 19:139–143

Frackowiak RSJ, Pozzilli C, Legg NJ, DuBoulay GH, Marshall J, Lenzi GL, Jones T (1981) Regional cerebral oxygen supply and utilization in dementia. A clinical and physiological study with oxygen-15 and positron tomography. Brain 104:753–788

Friedland RP, Budinger TF, Ganz E, Yano Y, Mathis CA, Koss B, Ober BA, Huesman RH, Derenzo SE (1983) Regional cerebral metabolic alterations in dementia of the Alzheimer type: positron emission tomography with (18F)-fluorodeoxyglucose. J Comput Assist Tomogr 7:590–598

Friston KJ, Frith CD, Liddle PF, Dolan RJ, Lammertsma AA, Frackowiak RSJ (1990) The relationship between global and local changes in PET scans. J Cereb Blood Flow Metab 10:458–466

Gibbs JM, Frackowiak RSJ, Legg NJ (1986) Regional cerebral blood flow and oxygen metabolism in dementia due to vascular disease. Gerontology 32[Suppl 1]:84–88

Hachinski VC, Iliff LD, Zilkha E, Duboulay GH, McAllister VL, Marshall J, Ross-Russell RW, Symon L (1975) Cerebral blood flow in dementia. Arch Neurol 32:632–637

Haxby JV, Grady CL, Duara R, Schlageter N, Berg G, Rapoport SI (1986) Neocortical metabolic abnormalities precede nonmemory cognitive defects in early Alzheimer's type dementia. Arch Neurol 43:882–885

Hayden MR, Hewitt J, Stoessl AJ, Clark C, Ammann W, Martin WRW (1987) The combined use of positron emission tomography and DNA polymorphisms for preclinical detection of Huntington's disease. Neurology 37:1441–1447

Heiss W-D, Hebold I, Klinkhammer P, Ziffling P, Szelies B, Pawlik G, Herholz K (1988) Effect of piracetam on cerebral glucose metabolism in Alzheimer's disease as measured by PET. J Cereb Blood Flow Metab 8:613–617

Heiss W-D, Herholz K, Böcher-Schwarz HG, Pawlik G, Wienhard K, Steinbrich W, Friedmann G (1986) PET, CT, and MR imaging in cerebrovascular disease. J Comput Assist Tomogr 10:903–911

Heiss W-D, Herholz K, Pawlik G, Hebold I, Klinkhammer P, Szelies B (1989) Positron emission tomography findings in dementia disorders: contributions to differential diagnosis and objectivizing of therapeutic effects. Keio J Med 38:111–135

Heiss W-D, Kessler J, Slansky I, Mielke R, Szelies B, Herholz K (1993) Longterm metabolic changes in Alzheimer's disease under various therapeutic interventions. J Cereb Blood Flow Metab 13[Suppl 1]:S5

Heiss W-D, Pawlik G, Herholz K, Göldner H, Wienhard K (1984) Regional kinetic constants and cerebral metabolic rate for glucose in normal human volunteers determined by dynamic positron emission tomography of (18F)-2-fluoro-2-deoxy-D-glucose. J Cereb Blood Flow Metab 4:212–223

Herholz K, Adams R, Kessler J, Szelies B, Grond M, Heiss W-D (1990) Criteria for the diagnosis of Alzheimer's disease with positron emission tomography. Dementia 1:156–164

Kamo H, McGeer PL, Harrop R, McGeer EG, Calne DB, Martin WRW, Pate BD (1987) Positron emission tomography and histopathology in Pick's disease. Neurology 37:439–445

Kessler J, Herholz K, Grond M, Heiss W-D (1991) Impaired metabolic activation in Alzheimer's disease: a PET study during continous visual recognition. Neuropsychologia 29:229–243

Klinkhammer P, Szelies B, Heiss W-D (1990) Effect of phosphatidylserine on cerebral glucose metabolism in Alzheimer's disease. Dementia 1:197–201

Kuhl DE, Metter EJ, Benson DF, Ashford JW, Riege WH, Fujikawa DG, Markham CH, Mazziotta JC, et al (1985) Similarities of cerebral glucose metablism in Alzheimer's and Parkinsonian dementia. J Cereb Blood Flow Metab 5[Suppl 1]:S169–S170

Kuhl DE, Metter EJ, Riege WH, Hawkins RA, Mazziotta JC, Phelps DE, Kling AS (1983) Local cerebral glucose utilization in elderly patients with depression, multiple infarct dementia, and Alzheimer's disease. J Cereb Blood Flow Metab 3[Suppl 1]:S494–495

Kuhl DE, Metter EJ, Riege WH, Markham CH (1984) Patterns of cerebral glucose utilization in Parkinson's disease and Huntington's disease. Ann Neurol 15[Suppl 1]:S119–S125

Mazziotta JC, Phelps ME, Pahl JJ, Huang S-C, Baxter LR, Riege WH, et al (1987) Reduced cerebral glucose metabolism in asymptomatic subjects at risk for Huntington's disease. N Engl J Med 316:357–362

McKhann G, Drachman D, Folstein MF, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease. Neurology 34:939–944

Mielke R, Herholz K, Grond M, Kessler J, Heiss W-D (1991) Differences of regional cerebral glucose metabolism between presenile and senile dementia of Alzheimer type. Neurobiol Aging 13:93–98

Nahmias C, Garnett ES, Firnau G, Lang A (1985) Striatal dopamine distribution in Parkinsonian patients during life. J Neurol Sci 69:223–230

Pawlik G (1988) Positron emission tomography and multiregional statistical analysis of brain function: from exploratory methods for single cases to inferential tests for multiple group designs. In: Willems JL, van Bemmel JH, Michel J (eds) Progress in computer-assisted function analysis. Elsevier, North-Holland, pp 401–408

Phelps ME, Huang SC, Hoffman EJ, Selin C, Sokoloff L, Kuhl DE (1979) Tomographic measurement of local cerebral glucose metabolic rate in humans with (F-18)-2-fluoro-2-deoxy-D-glucose: validation of method. Ann Neurol 6:371–388

Pietrzyk U, Herholz K, Heiss W-D (1990) Three-dimensional alignment of functional and morphological tomograms. J Comput Assist Tomogr 14:51–59

Reisberg B, Ferris SH, DeLeon MJ, Crook T (1982) The global deterioration scale for assessment of primary degenerative dementia. Am J Psychiatry 139: 1136–1139

Reivich M, Kuhl D, Wolf A, Greenberg J, Phelps M, Ido T, Casella V, Fowler J, Hoffman E, Alavi A, Som P, Sokoloff L (1979) The (18F)fluorodeoxyglucose method for the measurement of local cerebral glucose utilization in man. Circ Res 44:127–137

Riege WH, Metter EJ, Kuhl DE, Lanto AB, Small GW, Fujikawa DG, Mazziotta JC, Dorsey DA, Maltese A (1987) Alzheimer's disease: cerebral metabolic abnormalities coincide with early memory deficits. Soc Neurosci Abstr 13: 1628

Smith RC, Vroulis G, Johnson R, Morgan R (1984) Pharmacologic treatment of Alzheimer's type dementia: new approaches. Psychopharmacol Bull 20: 542–545

Sokoloff L, Reivich M, Kennedy C, Des Rosiers M-H, Patlak CS, Pettigrew KD, Sakurada O, Shinohara M (1977) The (14C)deoxyglucose method for the measurement of local cerebral glucose utilization: theory, procedure, and normal values in the conscious and anesthetized albino rat. J Neurochem 28:897–916

Szelies B, Herholz K, Pawlik G, Beil C, Wienhard K, Heiss W-D (1986) Zerebraler Glukosestoffwechsel bei präseniler Demenz vom Alzheimer-Typ – Verlaufskontrolle unter Therapie mit muskarinergem Cholinagonisten. Fortschr Neurol Psychiatr 54:364–373

Szelies B, Karenberg A (1986) Störungen des Glukosestoffwechsels bei Pick'scher Erkrankung. Fortschr Neurol Psychiatr 54:393–397

Wienhard K, Eriksson L, Grootoonk S, Casey M, Pietrzyk U, Heiss W-D (1992) Performance evaluation of the positron scanner ECAT EXACT. J Comput Assist Tomogr 16:804–813

Wienhard K, Pawlik G, Herholz K, Wagner R, Heiss W-D (1985) Estimation of local cerebral utilization by positron emission tomography of (18F)-2-fluoro-2-deoxy-D-glucose: a critical appraisal of optimization procedures. J Cereb Blood Flow Metab 5:115–125

Correspondence: Prof. Dr. W.-D. Heiss, Max-Planck-Institut für neurologische Forschung, Gleueler Strasse 50, D-50931 Köln, Federal Republic of Germany

Neurochemical investigations in patients with dementia of Alzheimer type and their clinical value

L. Frölich[1], **A. Dirr**[2], **M.E. Götz**[2], **Y. Taneli**[2], **J. Thome**[1], **K.P. Lesch**[2], **R. Ihl**[3], **T. Dierks**[1], **D. Blum-Degen**[2], **P. Riederer**[2], and **K. Maurer**[1]

[1] Department of Psychiatry I, University of Frankfurt/Main,
[2] Department of Psychiatry, University of Würzburg, and
[3] Department of Psychiatry, University of Düsseldorf, Federal Republic of Germany

Summary

The clinical diagnosis of dementia of Alzheimer type (DAT) relies on the identification of a dementia syndrome in the absence of other known etiologies, which results in a diagnostic validity of approximately 90 percent. Thus, the identification of a biological ante-mortem marker of DAT would be of great help. Investigations on post-mortem tissue have established several cascades of cell biological events in the affected brain, e.g. cholinergic degeneration, free oxygen radical toxicity, impairment of glucose metabolism, which we used as a rationale for testing the diagnosis utility of related parameters in DAT patients. Acetylcholine (ACh) and choline, and vitamin E in the CSF as well as neuroendocrine changes after a GHRH/CRH challenge and hormonal changes after an oral glucose tolerance in DAT patients were measured. For ACh concentration in CSF and neuroendocrine changes after GHRH as well as insulin release after OGTT, there were subtle changes from controls. The potential use of these parameters as diagnostic markers of Alzheimer's disease in the alive patient is discussed.

Introduction

Despite the great efforts to elucidate the pathophysiology of dementia of Alzheimer type (DAT) (Blass, 1993a), the clinical diagnosis of this disorder still remains a diagnosis of exclusion, which resides on the identification of a characteristic clinical syndrome, an unimpaired physical and neurological exam, and normal laboratory tests (McKhann et al., 1984; Kukull et al., 1990; Maurer et al., 1993). Modern techniques or neuroimaging provide some degree of diagnostic validity by typically

demonstrating a disturbed structure and function of the medial temporal lobe (including the hippocampal area) and the tertiary association areas (Friedland et al., 1985; Frölich et al., 1989; Jobst et al., 1992 a, b; Kesslak et al., 1991; Pearlson et al., 1992), but at the same time have provided evidence for heterogeneity of dementia of Alzheimer type e.g. demonstrating variability among the topography of the affected brain regions (Friedland, 1993). Furthermore, the reliability of the afore mentioned changes for an early diagnosis is not always established (Reed et al., 1989). The most rational approach for developing an in vivo biological marker of the disease arises from what is known about pathophysiologically relevant changes in the metabolism and neurochemistry of brain cells. However, since any direct biochemical determination of any parameter in the alive brain is impossible (unacceptability of a diagnostic brain biopsy in most countries), one is left with studying parameters of interest in compartments outside the primarily affected organ, which are thought to reflect changes in brain tissue with an acceptable degree of reliability. One of such compartments is the CSF, because 1. significant correlations between levels of neurotransmitters in the brain and the CSF can be demonstrated (Matsumoto et al., 1991) and 2. major confounding factors can be controlled to an acceptable extent (Frölich et al., 1991). Another interesting approach is studying the function of the hypothalamus-pituitary-adrenal axis, which may be regarded a window to the brain, at least in some functional psychiatric disorders (Lesch and Rupprecht, 1990). Yet another approach relies on the assumption that certain general disturbances of cell metabolism may exist in DAT, caused by a general pathobiochemical abnormality (Blass, 1993b).

In line with these assumptions and starting from three pathogenetic hypotheses of neuronal degeneration in DAT. we have carried out four sets of in vivo investigations in DAT patients. 1. Following the cholinergic hypothesis of DAT, we have measured the concentration of acetylcholine (ACh) and choline in the CSF. 2. Following the free oxygen radical hypothesis of neuronal degeneration, we have measured the concentration of vitamin E (α- and g-tocopherol) in paired samples of CSF and serum. 3. After a bolus injection of GHRH or CRH, changes in the serum concentration of growth hormone, ACTH and cortisol over time were measured. 4. Following the hypothesis of a primary disturbance of glucose metabolism, changes in serum glucose and insulin concentration after an oral glucose tolerance test were measured over time.

Methods

Patients

All investigations were performed on patients with dementia from the Department of Psychiatry, University of Würzburg. These patients fulfilled the NINCADS/ADRDA criteria for probable DAT (McKhann et al., 1984). Diagnostic assessment included history, physical, neurological, and psychiatric examination and routine laboratory tests (incl. thyroid hormone levels, vitamin B12 and folate). The modified Hachinski ischemic score (score <4) was used to exclude multi-infarct dementia (Hachinski et al., 1975). Further selection was based on CT scans in all patients showing only cerebral atrophy, ventricular dilatation and no more than 1 lacunar infarction, if abnormal. In no case, there were territorial infarctions. All patients were investigated with a battery of neuropsychological tests for cognitive functions, language, apraxia, agnosia, visuspatial abilities, mood and behavioral changes (short performance test, mini mental state examination (MMS), Alzheimer disease assesssment scale, brief cognitive rating scale) (Ihl et al., 1992). CSF studies were performed on hospitalized patients, while the patients for hormone studies were not admitted to the hospital.

Controls

For CSF studies, hospitalized neurological patients were selected as the control group. These patients originally were admitted to the hospital with nonspecific complaints such as dizziness, headache or nonspecific complaints. None of the patients showed clinical signs of dementia by history and psychiatric exam. None of the patients had previously had an operation on or near the central nervous system, e.g. lumbar disc surgery. Standard neurological examination showed no indication of pathology. The results from a CT scan were within normal limits, except for evidence for brain atrophy in some patients. CSF analysis showed a total white cell count below 5 cells/ul, each CSF sample was clear and colorless, and isoelectric focusing on polyacrylamide gels revealed no evidence for oligoclonal bands. For hormone studies, age-matched healthy non-hospitalized volunteers were selected. None of these subjects showed clinical signs of dementia by history and psychiatric screening, either.

CSF analysis

The CSF was taken during routine lumbar puncture performed for diagnosis purposes in the morning after an over-night fasting. Blood samples were drawn at the same time and assayed simultaneously. Routine CSF analysis (cytology, isoelectric focusing, microbiological investigations, viral titers) did not reveal signs of a chronic inflammatory process. For ACh determination, 10 ml CSF was dripped into ice-chilled tubes containing 2 ml perchloracetic acid to inhibit cholinesterase activity in the CSF. A centrifuged aliquot of the supernatant was concentrated by a solid phase extraction procedure and injected into an HPLC

system with electrochemical detection modified after Stadler and Nesselhut (1986) and Okuyama et al. (1988). For vitamin E determination, 1 ml CSF was given into tubes containing 1 ml ethanol; after extraction and concentration with an ethanol/hexane phase, an aliquot of the lipophilic phase was injected into and HPLC system with electrochemical detection modified after Vatasarry et al. (1987).

Hormone studies

For GHRH challenge, all subjects received 50 < synthetic human GHRH-44 amide and 100 μg synthetic human CRH-41 as an i.v. bolus dose at bed rest in the morning. Hormone levels were measured before, and at 0, 15, 30, 45, 60, 90 and 120 min. after injection. Standard radioimmunoassays were employed for the determination of GH, ACTH and cortisol analyses. For the oral glucose tolerance test, all subjects received 100 mg glucose in the morning after a night fasting. Blood was drawn before, 30, 60, 90, 120, 180 and 240 min. after the glucose load. Glucose and insulin levels were determined with standard enzymatic tests and radioimmunoassays.

Data analysis

All data are presented as mean ± standard error of mean and analysed using a non-parametric statistical test (Mann-Whitney U test).

Results

The present HPLC system with solid phase extraction and electrochemical detection had a detection limit of 1 pmol/ml CSF both for ACh and choline (data for recovery and sensitivity not shown). In 11 DAT patients, 3 samples showed ACh values above detection limit with 3.9 ± 1.3 pmol/ml CSF (mean value ± SEM). In 15 controls, 12 values were above detection limit with 5.7 ± 1.3 pmol/ml CSF. These group differences were not statistically significant. Choline values were 2169 ± 344 pmol/ml CSF for 11 DAT patients and 2145 ± 221 pmol/ml CSF for 15 controls, respectively. These differences were not statistically significant, either ($p < 0.1$).

In the second study, alpha- and gamma-tocopherol were determined in paired samples of serum and CSF. In 8 DAT patients, alpha-tocopherol showed a concentration of 13.6 ± 5.0 μg/ml serum and 3.7 ± 1.4 ng/ml CSF. In 12 controls, alpha-tocopherol showed a concentration of 11.3 ± 3.3 μg/ml serum and 6.7 ± 2.1 ng/ml CSF. Gamma-tocopherol showed a concentration of 8.5 ± 0.9 μg/ml serum and 4.3 ± 3.0 ng/ml CSF in DAT patients, and a concentration of 9.5 ± 3.2 μg/ml serum and 5.8 ± 3.5 ng/ml CSF in controls. None of the

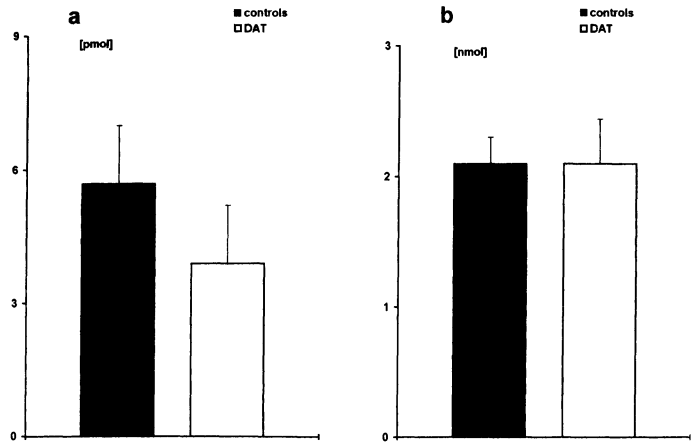

Fig. 1. **a** Acetylcholine concentration in CSF (N = 3 DAT, N = 8 controls) and **b** choline concentration in CSF (N = 11 DAT, N = 15 controls). Values are presented as mean ± SEM. Neither group means of detectable levels nor the frequency of samples with detectable amounts of ACh to total sample number (11 samples in DAT; 15 samples in controls) were significantly different between DAT and control group

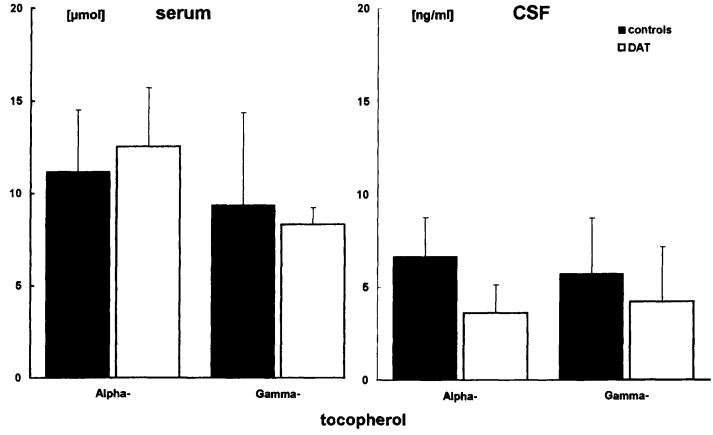

Fig. 2. Alpha- and gamma-tocopherol concentration in serum and CSF of 8 DAT patients and 12 controls. Values are presented as mean ± SEM. There were no significant differences between DAT and control group

differences between DAT and control group were statistically significant ($P < 0.1$).

Compared with 10 age-matched controls, 10 patients with DAT showed significantly attenuated GH responses (area under the curve:

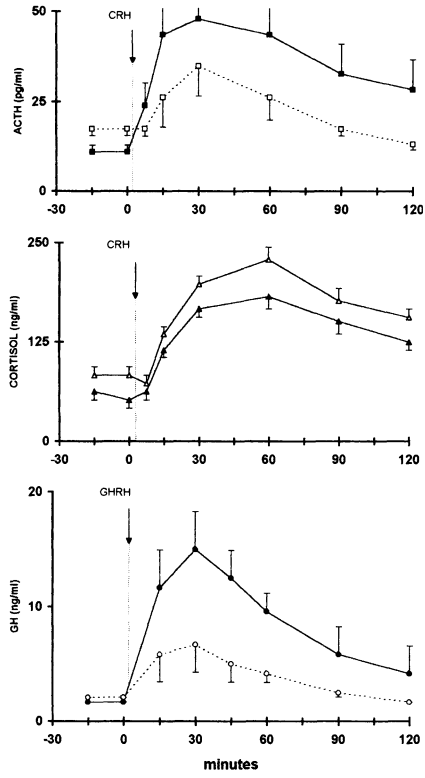

Fig. 3. Time course of GH responses to GHRH and CRH-induced ACTH/cortisol release in 10 DAT patients and 10 controls. Values are presented as mean ± SEM. Both GH and ACTH responses as measured by areas under the curve were significantly reduced in DAT patients ($p < 0.05$ by Mann-Whitney U test)

851 ± 232 vs. 361 ± 114 ng · min/ml; $p < 0.05$) to GHRH; and decreased ACTH (area under the curve 3136 ± 885 vs. 1431 ± 261 pg · min/ml; $p < 0.05$), but normal cortisol release following CRH. Basal GH, ACTH and cortisol levels in DAT were not significantly different from control values.

15 DAT patients showed a significantly reduced serum glucose concentration (88 ± 2.7 vs. 101 ± 4.9 mg/di; $p < 0.05$) before an oral glucose tolerance test (OGTT), compared to 10 age-matched controls. However, glucose levels after OGTT were not significantly different (area under the curve: 3250 ± 277 vs. 3331 ± 271 mg · min/di; $P < 0.1$). Basal insulin concentrations were not significantly different, but insulin levels 120 min. after OGTT tended to be increased (137 ± 17 vs, 100 ± 1.5 ng/ml; $p < 0.1$).

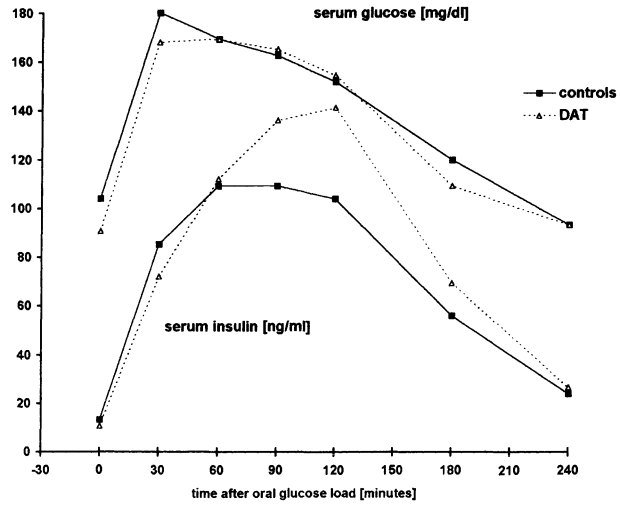

Fig. 4. Time course of glucose and insulin responses to an oral glucose tolerance test in 15 DAT patients and 10 controls. Values are presented as mean ± SEM. Basal serum glucose concentration was significantly decreased in DAT patients ($p < 0.05$ by Mann-Whitney U test), while insulin concentration showed a trend to increase at 120 min. after glucose load ($p < 0.1$ by Mann-Whitney U test)

Discussion

The most consistent finding in neurochemical studies of DAT is the large reduction of choline acetyltransferase (CAT) activity in several brain areas (for review see Gsell et al., 1993), which is attributed to the degeneration of cholinergic neurons in the nucleus basalis of Meynert (Arendt et al., 1983; McGeer et al., 1984). This loss of CAT activity is correlated with both the number of neuritic plaques and the severity of dementia as measured by cognitive tests before death (Blessed et al., 1968). To determine the functional activity of the cholinergic neurotransmitter system in vivo, a direct measurement of the neurotransmitter acetylcholine in the CSF might be more sensitive than measuring the activity of the synthetic enzyme CAT. However, in our investigations, several samples of CSF in both DAT patients and neurological controls were below the detection limit for ACh, thus the results escaped from statistical significance. Similar results have been obtained by others (Teelken et al., 1991). Choline levels were unchanged and in the range reported by other (Kumar et al., 1989; Schapiro et al., 1990), although there have also been reports of increased choline levels in the CSF in DAT (Eible et al., 1989). ion earlier investigations, the activity of both CAT activity and acetylcholine esterase (ACHE) in the CSF were found

to be reduced to a variable extent (Atack et al., 1983; Kumar et al., 1989; Navaratnam et al., 1991; Shen et al., 1993), although it has been argued that normal human CSF does not contain CAT (DeKosky et al., 1989). In the CSF of DAT patients, numerous neurochemical parameters have been reported to be altered (Alom et al., 1990; Kaye et al., 1988; Pomara et al., 1989). Neurochemical parameteres of the cholinergic neurotransmitter system have been reported to be altered in other body compartments outside the CSF (Adem et al., 1986; Foley et al., 1988). Interesting, the variability of heart frequency, which is related to autonomic parasympathetic (=cholinergic) cardiac innervation, has been shown to be altered in DAT (Aharon-Peretz et al., 1992), providing a potential neurophysiological marker. It might be also of interest to study other parameters of the autonomic cholinergic system in DAT which are known to be altered by e.g. a pharmacological cholinergic blockade (Kristensen et al., 1989). Similarly, neuroendocrine parameters in serum which can be modulated by a pharmacological cholinergic stimulation, appear to be changed in DAT patients (Raskind et al., 1989). Our neuroendocrine studies demonstrated a blunted GH response to GHRH, a blunted ACTH response to CRH and a discrete hypercortisolism in DAT, which might also be related to central cholinergic deficits or a generally decreased adaptation to stress situations (Lesch et al., 1990).

Oxidative stress has been suggested to be an etiological factor in several neurodegenerative diseases, e.g. Parkinson's disease (Götz et al., 1990). An uncoupling of oxidative glucose metabolism (Sims et al., 1989) or other changes in mitochondrial metabolism (Blass et al., 1988; Wallace, 1992) as have been reported to occur in DAT, might lead to increased formation of free oxygen radicals (Halliwell, 1992f). Since radical scavenging enzymes only appear slightly altered in DAT (Gsell et al., 1994), a decreased availability of vitamin E, the major lipid-soluble radical scavenging substance, may be hypothesized in DAT. However, our results on unchanged vitamin E levels in CSF in DAT as well as those of others on undisturbed vitamin E levels in brain tissue (Metcalfe et al., 1989; Adams et al., 1991), do not support this hypothesis. Our control values on alpha- and gamma-tocopherol in CSF were similar to those of an earlier investigation, where HPLC with fluorometric determination was used (Vatassery et al., 1991).

Following the hypothesis of a defect in cellular brain glucose and energy metabolism (Hoyer et al., 1988), an increased concentration of insulin in the CSF and an abnormal (=hyperinsulinemic) response to a glucose tolerance test has been shown (Fujisawa et al., 1991). This could only partially be supported by our preliminary data on a oral glucose tolerance test in a highly selected group of DAT patients. Similar changes had been reported earlier in a retrospective analysis of patients with degenerative dementia (Bucht et al., 1983), but could not

be reproduced in another small set of DAT patients (Winograd et al., 1991; Kilander et al., 1992).

All these pathobiochemical changes in Alzheimer patients reveal group differences from controls, but do not in a single patient provide clear-cut deviations from normal values, thus no diagnostically relevant ante-mortem biological marker of DAT has been obtained, yet (Hollander et al., 1986; Cutler et al., 1988).

References

Adams Jr JD, Klaidman LK, Odunze IN, Shen HC, Miller CA (1991) Alzheimer's and Parkinson's disease – brain levels of glutathione, glutathione disulfide, and Vitamin E. Mol Chem Neuropathol 14:213–226

Adem A, Nordberg, A, Bucht, G, Winblad B (1986) Extraneuronal cholinergic markers in Alzheimer's and Parkinson's disease. Prog Neurosychopharmocol Biol Psychiatry 10:247–257

Aharon-Peretz J, Harel T, Revach M, Ben-Haim SA (1992) Increased sympathetic and decreased parasympathetic cardiac innervation in patients with Alzheimer's disease. Arch Neurol 49:919–922

Alom J, Galard R, Catalan R, Castellanos JM, Schwartz S, Tolosa E (1990) Cerebrospinal fluid neuropeptide Y in Alzheimer's disease. Eur Neurol 30:207–210

Arendt T, Bigl V, Arendt A, Tennstedt A (1983) Loss of neurons in the nucleus basalis of Meynert in Alzheimer's disease, paralysis agitans and Korsakoff's disease. Acta Neuropathol 61:101–108

Atack JR, Perry EK, Bonham JR, Perry RH, Tomlinson BE, Blessed G, Fairbairn A (1983) Molecular forms of acetylcholinesterase in senile dementia of ALzheimer type: selective loss of the intermediate (10S) form. Neurosci Lett 40:199–204

Blass JP (1993a) Pathophysiology of the Alzheimer's syndrome. Neurology 43 [Suppl 4]:25–38

Blass JP (1993b) Metabolic alterations common to neural and non-neural cells in Alzheimer's disease. Hippocampus 3:45–54

Blass JP, Sheu RK, Cedarbaum JM (1988) Energy metabolism in disorders of the nervous system. Rev Neurol (Paris) 144:543–563

Blessed G, Tomlinson BE, Roth M (1968) The association between quantitative measures of dementia and of senile change in the cerebral grey matter of elderly subjects. Br J Psychiatry 114:797–811

Bucht G, Adolfsson R, Lithner F, Winblad B (1983) Changes in blood glucose and insulin secretion in patients with senile dementia of Alzheimer type. Acta Med Scand 213:387–392

Cutler NR (1988) Utility of biological markers in the evaluation and diagnosis of Alzheimer's disease. Brain Dysfunct 1:12–31

DeKosky St T, Scheff St W, Hackney CG (1989) Acetylcholine synthesis in human CSF: implications for study of central cholinergic metabolism. Neurochem Res 14:191–196

Elble R, Giacobini E, Higgins C (1989) Choline levels are increased in cerebrospinal fluid of Alzheimer patients. Neurobiol Aging 10:45–50

Foley P, Bradford HF, Docherty M, Fillit H, Luine VN, McEwen B, Bucht G, Winblad B, Hardy J (1988) Evidence for the presence of antibodies to cholinergic neurons in the serum of patients with Alzheimer's disease. J Neurol 235:466–471

Friedland RP (1993) Alzheimer's disease: clinical features and differential diagnosis. Neurology 43[Suppl 4]:45–51

Friedland RP, Brun A, Budinger TF (1985) Pathological and positron emission tomographic correlations in Alzheimer's disease. Lancet i:228

Frölich L, Eilles C, Ihl R, Maurer K, Lanczik M (1989) Stage-dependent reductions of regional cerebral blood flow measured by HMPAO-SPECT in dementia of Alzheimer type. Psychiatry Res 29:347–350

Frölich L, Kornhuber J, Ihl R, Fritze J, Maurer K, Riederer P (1991) Integrity of the blood-CSF barrier in dementia of Alzheimer type: CSF/serum ratios of albumin and IgG. Eur Arch Psychiatry Clin Neurosci 240:363–366

Fujisawa Y, Sasaki K, Akiyama K (1991) Increased insulin levels after OGTT load in peripheral blood and cerebrospinal fluid of patients with dementia of Alzheimer type. Biol Psychiatry 30:1219–1228

Götz ME, Freyberger A, Riederer P (1990) Oxidative stress: a role in the pathogenesis of Parkinson's disease. J Neural Transm [Suppl 29]:241–249

Gsell W, Moll G, Sofic E, Riederer P (1993) Cholinergic and monoaminergic neurotransmitter system in patients with Alzheimer's disease and senile dementia of the Alzheimer type: a critical evaluation. In: Maurer K (ed) Dementias, neurochemistry, neuropathology, neuroimaging, neurpsychology and genetics. Vieweg, Braunschweig, pp 25–51

Gsell W, Conrad R, Hickethier M, Sofic E, Frölich L, Wichart I, Jellinger K, Moll G, Ransmayr G, Beckmann H, Riederer P (1994) Decrease in catalase activity and inducibility of superoxide dismutase activity in brains of patients with senile dementia of Alzheimer type. Neurochem (in press)

Hachinski V, Iliff LD, Zilkha E, et al (1975) Cerebral blood flow in dementia. Arch Neurol 32:632–637

Halliwell B (1992) Reactive oxygen species and the central nervous system. J Neurochem 59:1609–1623

Hollander E, Mohs RC, Davis KL (1986) Antemortem markers of Alzheimer's disease. Neurobiol Aging 7: 367–387

Hoyer S, Oesterreich K, Wagner O (1988) Glucose metabolism as the site of the primary abnormality in early-onset dementia of Alzheimer type? J Neurol 235:143–148

Ihl R, Frölich L, Dierks T, Martin E, Maurer K (1992) Differential validity of psychometric tests in dementia of the Alzheimer type. Psychiatry Res 44:93–106

Jobst KA, Smith AD, Szatmari M, Molyneux A, Esiri ME, King E, Jaskowski A, McDonald B, Wald N (1992) Detection in life of confirmed Alzheimer's disease using a simple measurement of medial temporal lobe atrophy by computed tomography. Lancet 340:1179–1183

Jobst KA, Smith AD, Barker CS, Wear A, King EM, Smith A, Anslow PA, Molyneux AJ, Shepstone BJ, Soper N, Holmes KA, Robinson JR, Hope RA, Oppenheimer C, Brockbank K, McDonald B (1992) Association of atrophy of the medial temporal lobe with reduced blood flow in the posterior parietotemporal cortex in patients with a clinical and pathological diagnosis of Alzheimer's disease. J Neurol Neurosurg Psychiatry 55:190–194

Kaye JA, May C, Atack JR, Daly E, Sweeney DL, Beal MF, Kaufman S, Milstien S, Friedland RP, Rapoport SI (1988) Cerebrospinal fluid neurochemistry in the myoclonic subtype of Alzheimer's disease. Ann Neurol 24:647–650

Kesslak JP, Nalcioglu O, Cotman CW (1991) Quantification of magnetic resonance scans for hippocampal and parahippocampal atrophy in Alzheimer's disease. Neurology 41:51–54

Kilander L, Boberg M, Lithell H (1992) Peripheral glucose metabolism and insulin sensitivity in Alzheimer's disease (poster, unpublished)

Kristensen E, Jakobsen J, Bartels U, Vestergaard P (1989) Cholinergic dysfunction of heart, pupil, salivary glands, and urinary bladder in healthy volunteers during long-term treatment with clomipramine. Psychopharmacology 98:398–402

Kukull WA, Larson EB, Reifler BV, Lampe TH, Yerby MS, Hughes JP (1990) The validity of 3 clinical diagnostic criteria for Alzheimer's disease. Neurology 40:1364–1369

Kumar V, Giacobini E, Markwell S (1989) CSF choline and acetylcholinesterase in early-onset vs. late-onset Alzheimer's disease patients. Acta Neurol Scand 80:461–466

Lesch KP, Rupprecht R (1990) Psychoneuroendocrine research in depression. II. Hormone responses to releasing hormones as a probe for hypothalamic-pituitary-endorgan dysfunction. J Neural Transm 75:179–194

Lesch KP, Ihl R, Frölich L, Rupprecht R, Müller U, Schulte H-M, Maurer K (1990) Endocrine responses to growth hormone releasing hormone and corticotriopin releasing hormone in early-onset Alzheimer's disease. Psychiatry Res 33:107–112

Matsumoto M, Togashi H, Yoshioka M, Morii K, Hirokami M, Tochihara M, Ikeda T, Saito Y, Saito H (1991) Significant correlations between cerebrospinal fluid and brain levels of norepindephrine, serotonin and acetylcholine in anesthetized rats. Life Sci 48:823–829

Maurer K, Ihl R, Frölich L (1993) Alzheimer. Springer, Berlin Heidelberg New York Tokyo

McGeer PL, Mc Geer EG, Suzuki J, Dolman CE, Nagai T (1984) Aging, Alzheimer's disease and the cholinergic system of the basal forebrain. Neurology 34:741–745

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's disease. Neurology 34:939–944

Metcalfe T, Bowen DM, Muller DPR (1989) Vitamine E concentrations in human brain of patients with Alzheimer's disease, fetuses with Down's syndrome, centenarians and controls. Neurochem Res 14:1209–1212

Navaratnam DS, Priddle JD, McDonald B, Esiri MM, Robbinson JR, Smith AD (1991) Anomalous molecular form of acetylcholinesterase in cerebrospinal fluid in histologically diagnosed Alzheimer's disease. Lancet 337:447–449

Okuyama S, Ikeda Y (1988) Determination of acetylcholine and choline in human cerebrospinal fluid using high-performance liquid chromatography combined with an immobilized enzyme reactor: ageing-induced change of acetylcholine level. J Chromatogr 431:389–394

Pearlson GD, Harris GJ, Power RE, Barta PE, Camargo E, Chase GA, Noga JT, Tune LE (1992) Quantitative changes in mesial temporal volume, regional

cerebral blood flow and cognition in Alzheimer's disease. Arch Gen Psychiatry 49:402–408

Pomara N, Singh R, Deptula D, LeWitt PA, Bissette G, Stanley M, Nemeroff CB (1989) CSF Corticotrophin-Releasing Factor (CRF) in Alzheimer's disease: its relationship to severity of dementia and monoamine metabolites. Biol Psychiatry 26:500–504

Raskind MA, Peskind ER, Veith RC, Risse St C, Lampe TH, Borson S, Gumbrecht G, Dorsa DM (1989) Neuroendocrine responses to physostigmine in Alzheimer's disease. Arch Gen Psychiatry 46:535–540

Reed BR, Jagust WJ, Seab Ph, Ober BA (1989) Memory and regional cerebral blood flow in mildly symptomatic Alzheimer's disease. Neurology 39:1537–1539

Schapiro MB, Atack JR, Hanin I, Max C, Haxby JV, Rapoport St I (1990) Lumbar cerebrospinal fluid choline in healthy aging and in Down's syndrome. Arch Neurol 47:977–980

Shen ZX, Ding Q, Wei CZ, Ding MC, Meng JM (1993) CSF Cholinesterase in early-onset and late-onset Alzheimer's disease and multi-infarct dementia of Chinese patients. Acta Neurol Scand 87:19–24

Sims NR, Finegan JM, Blass JP, Bowen DM, Neary D (1989) Mitochondrial function in brain tissue in primary degenerative dementias. Brain Res 436:30–38

Stadler H, Nesslhut T (1986) Simple and rapid measurement of acetylcholine and choline by HPLC and electrochemical detection. Neurochem Int 9:127–129

Teelken AW, Schuring HF, Trieling WB, Damsma G (1991) Measurement of acetylcholine and choline in cerebrospinal fluid by means of HPLC (poster, unpublished)

Vatassery GT, Smith WE (1987) Detection of alpha-tocopherol quinone (vitamin E quinone) in human serum, platelets and red cell membrane samples. Anal Biochem 167:411–417

Vatassery GT, Nelson MJ, Maletta GJ, Kuskowski MA (1991) Vitamin E (tocopherols) in human cerebrospinal fluid. Am J Clin Nutr 53:95–99

Wallace DC (1992) Mitochondrial genetics: a paradigm for aging and degenerative disease? Science 256:628–632

Winograd C-H, Jacobson D, Minkoff J-R, Peabody C-A, Taylor B-S, Widirow L, Yesavage J-A (1991) Blood glucose and insulin response in patients with senile dementia of the Alzheimer's type. Biol Psychiatry 30:507–511

Correspondence: Dr. L. Frölich, Department of Psychiatry I, J. W. Goethe University of Frankfurt/Main, Theodor-Stern-Kai, D-60596 Frankfurt/Main, Federal Republic of Germany

Different methodological approaches for the construction of a therapy sensitive ADL scale for the assessment of Alzheimer patients

M. Stemmler[1], **K.-C. Steinwachs**[2], **H. Lehfeld**[1], **J. Jentzsch**[2], **K. Tritt**[1], **F.W. Hulla**[3], and **H. Erzigkeit**[1]

[1] Psychiatrische Universitäts-Klinik and [2] Bezirkskrankenhaus, Erlangen, and [3] BAYER AG, Leverkusen/Wuppertal, Federal Republic of Germany

Summary

The purpose of this paper was the presentation of the work of the international ADL project which aims to construct a therapy sensitive ADL scale for cognitively impaired elderly or demented patients. No final scale was presented, rather different ways for generating ADL items were introduced. Different methodological approaches were applied because no existing ADL or IADL scale meets the various goals of the international ADL project. By surveying existing scales or assessment tools for measuring daily life behaviour it was recognized that no traditional method of scale construction seems appropriate. Instead, different methodological approaches were adapted in order to meet the high demands for a newly to be constructed internationally valid and therapy sensitive ADL scale for mild to moderate dementia. The scale construction is still in progress. A first scale is expected to be tested by the end of 1994. An international validation in European and American research centers (including one center in Russia) is planned.

Introduction

In this paper, the work of the international ADL project, which, – applies different methods for constructing a therapy sensitive scale for the assessment of activities of daily living (ADL) in cognitively impaired elderly or demented patients – will be presented. After giving a brief overview of the need for measuring daily life activities in demented patients, the different methods for generating therapy sensitive ADL items will be outlined.

Recommendations for the efficacy proof of antidementia drugs in clinical trials suggest the provision of evidence on independent assess-

ment levels such as the psychopathological, the psychometric, and the behavioural level with regard to activities of daily living (Kanowski et al., 1990). In the same way the "CPMP working party on efficacy of medicinal products" (1993) stated in its "Notes for Guidance" on antidementia medicinal products that besides to improvement in cognition and overall clinical impression antidementia claims should be supported by "improvement in behaviour closely connected with activities of daily living" (p. 84).

Whereas the psychopathological and the psychometric assessment of cognitively impaired patients seems sufficiently established, a general lack of internationally accepted and validated tools for assessing the effects of therapeutic measures on activities of daily living (ADL) in the elderly must be acknowledged. This is due to the fact that traditional methods of ADL assessment (e.g., Katz et al., 1963; Lawton and Brody, 1969) have mainly focused on the functional dimension of self-care and self-maintenance. Due to their emphasis on basic self-care activities, traditional ADL scales cannot easily be adapted to assess the full range of change in terms of daily life behaviour, which is related to the cognitive impairment of the patient in the course of senile dementia. Thus, in order to evaluate therapeutic effects, especially in mildly or moderately demented patients, a broader conceptualization of "activities" of daily living needs to be applied. This conceptualization should also enable, in addition to normative measurements, a differential documentation of the patients's life with idiographic components concerning aspects of autonomy or quality of life (Erzigkeit, 1989).

The ADL project[1] pursues the task of developing "for specific use in pharmacologic trials in dementia an expeditious, sensitive and cross-culturally valid assessment of functional capacities relevant for maintaining one's autonomy and ability to structure everyday life" (p. 3; Sclan, 1993). The pursuit of this goal, however, is laborious and complicated. This is in part due to the fact that the development of such an ADL scale faces a crucial problem: Appropriate ADL scales for the stages of mild to moderate dementia and for the assessment of the efficacy of antidementia drugs need to be highly sensitive to detect even small effect sizes induced by therapeutic intervention. But, at the

[1] The international ADL project was founded by H. Erzigkeit in 1990 and is now managed and coordinated by F. W. Hulla of BAYER AG, Leverkusen, Germany. The ADL project consists momentarily of four international workgroups: (1) The New York workgroup (B. Reisberg and S. G. Sclan), (2) the Chicago workgroup (S. Finkel), (3) the Berlin workgroup (S. Kanowski and colleagues), and the Erlangen workgroup (H. Erzigkeit and colleagues). The establishment of a fifth workgroup at the Bekhterev Institue in St. Petersburg, Russia (M. Kabanov, V. Wied and collegues) is in progress. The ADL workgroups are coordinated and regulated by a steering committee (chair: B. Reisberg) which consists of the heased of each workgroup, F. W. Hulla, and four additional experts from the field of psychiatry and psychogeriatrics: M. Bergener (Bergisch Gladbach), I. Hindmarch (University of Surrey), J. E. Overall (University of Houston), and L. Poon (University of Georgia)

same time the interindividual variability is large in cognition, cognitive styles, compensatory strategies, and other psychosocial factors in persons at the early stage of dementia (Poon, 1993). Thus, the difficulty of employing ADL scales for early detection is that when the highest level of measurement is needed, the ADL scale sensitivity is at its lowest level (Poon, 1993).

This presents a problem in view of the high demand on such a new instrument: The instrument should, of course, meet all basic psychometric requirements like objectivity, reliability, and validity. The instrument should also be relevant for everyday behaviour and be nonspecific to gender. In addition, the assessment tool should be internationally validated. Finally, the measure should not only be based on a deficit model, but should assess functional abilities such as adaptation and coping mechanisms (Erzigkeit et al., 1993).

Materials and methods

In order to achieve the above mentioned goals, the ADL project applies a multimethod approach. Different workgroups were established to pursue these methodological approaches; whereby each workgroup has the main responsibility for at least one methodological approach, one approach can be followed by more than one workgroup (see Table 1).

(1) One workgroup was responsible mainly for *surveying existing ADL scales* with regard to their usefulness concerning the aims of the ADL project.
(2) This survey resulted in the construction of a huge and diverse item pool consisting of 2626 items. The item pool was prepared for an evaluation by experienced clinicians (i.e., *clinician rating of the item pool*).
(3) In addition to the above mentioned methodological approaches, the clinician rating workgroup formulated items which described cognitive deficits in combination with impairments of activities of daily living. For this approach items of the item pool appropriate for mild and moderate dementia were used as a guide. Thereby, it was attempted to encompass all cognitive deficits listed in the diagnostic criteria of DSM-III-R and ICD 10 (i.e., *item formulation based on diagnostic criteria and items of the item pool appropriate for mild and moderate dementia*).
(4) *Patient-based interview* workgroups were established to conduct qualita-

Table 1. Different methods for ADL item generation

Methods:
(1) Survey of existing ADL scales (item pool consisting of 2626 items)
(2) Clinician rating of the item pool
(3) Item formulation based on diagnostic criteria and items of the item pool appropriate for mild and moderate dementia
(4) Patient-based interviews
(5) Expert-interviews

tive interviews with very mildly to moderately impaired patients and their collaterals.

(5) The *expert-interview* workgroup conducted interviews with experts from the fields of geriatrics and psychogeriatrics.

One workgroup is still in the planning stage: The experimental test situation workgroup (i.e., *creation of an objective test situation*) which will aim to construct and objective test situation for the observation and assessment of impairments in ADL and IADL.

Ad (1). For the survey of existing ADL scales the data bank "Medline" was used to identify published articles referring to such scales. Keywords such as "activities of daily living", "quality of life", "Alzheimer's Disease", "dementia", or "assessment" were entered to identify relevant scales. All articles found were retrieved, reviewed, and appropriate scales were extracted. Copies of these scales were obtained from the author(s), publisher(s), or organization(s). A total of 92 scales were collected by these means, consisting of 64 observer-rating scales and 28 self-rating scales. Of the 92 scales only 12 were specific ADL or IADL scales (see Table 2).

In order to get a preliminary overview of the total item pool, the items were sorted into 11 major categories. The categories were either suggested by the scale constructors or, when not available, emerged from the content of the items. The number of items in each category can be seen in Table 3.

The largest number of items (n = 603) belonged to the category of ADL and described traditional ADL aspects related to self-care. The second largest category containing n = 537 items was "cognition". About equal in size and still relatively large were the categories of "social relations" (n = 278) and "psychiatric symptoms" (n = 276). Of moderate size were the categories of "depression" (n = 245), "instrumental activities of daily living" (n = 220), "general state of health" (n = 211), and "life satisfaction" (n = 201). Only a small number of items assessed "economic resources" (n = 15), "self-image" (n = 27), and "locus of control" (n = 13). In brief, a clear predominance of the items assessed activities of daily living (ADL).

Ad (2). Based on the discussion of the 4th Meeting of the Methodenforum workgroup (Protokollmappe des 4. Erlanger Methodenforums, 1990), a procedure was developed to evaluate the relevance of the items by means of ratings carried out by experienced clinicians. N = 135 clinicians from 13 different institutions (i.e., hospitals or universities) took part in this study. Each clinician was presented with a random selection of 100 items from the consolidated file. In order to avoid anchor effects caused by the sequence of the item presentation, the items were presented to each clinician in a different

Table 2. Construction of the item pool

The databank MEDLINE was used to identify published articles referring to ADL scales.
Results of MEDLINE research:
p12 scales which assess solely ADL or IADL
p71 scales which contain at least one ADL or IADL subscale
p9 scales which contain ADL or IADL related items
92 scales (deadline: summer 1992)

Table 3. Categories of items resulting from item pool (total number of items = 2626)

Item class or item category	Defining properties of item class or item category	Number of items
Activities or daily living (ADL)	basic self-care activities (e.g., feeding, bathing, continence)	603
Cognition	short and long-term memory, concentration, orientation, logical thinking	537
Social relations	communication, relationships to friends and family, role behavior	278
Psychiatric symptoms	autism, hostility, aggressive behavior, emotional lability, paranoia	276
Depression	dysphoria, reduced motivation, lack of initiative	245
Instrumental activities of daily living (IADL)	complex daily living activities (e.g., using a telephone, shopping, housework, etc.)	220
General state of health	somatic fitness, aspects of multimorbidity, physical symptoms	211
Life satisfaction	subjective well-being, general contentment	201
Self-image	global self-estimation, self-esteem, achievement orientation	27
Economic resources	financial security, financial resources, social status	15
Locus of control	subjective estimation of the origins of success and failure, dependency or judgements of others	13

randomized order. Each of the 2626 items was rated by up to five clinicians (although the item was assigned to five raters, in a minority of cases one or two responses were missing). As part of the assessment procedure the clinicians were informed of the goal of the ADL project. The clinicians were instructed to base their judgements solely on their clinical experience and to focus on the content and not on the particular structure of the item (i.e., yes/no question, Likert-type scale). Each item was rated according to the following aspects: (1) therapy sensitivity, (2) clinical relevance, (3) adequacy for application to different degree of severity (mild/moderate/severe), and (4) the item's general appropriateness for inclusion in an ADL scale.

Statistical criteria were used in an attempt to reduce the item pool. For this reduction, at least 4 out of 5 raters had to evaluate a listed item as being generally useful for inclusion in an ADL scale. The items also had to have high ratings for therapy sensitivity and for clinical relevance (i.e., smaller or equal to 4 on a 6-point scale with smaller numbers representing more clinical relevance or higher therapy sensitivity). This procedure resulted in a reduced set of 377 items. The listing of the reduced item pool was used in an attempt to place specific symptoms and behaviours along a scale of increasing severity of dementia. For this purpose, the items were put in order according to their ratings for "adequacy for application to different degrees of severity (mild/moderate/severe)" of dementia. It was recognized that many of the 2626 items

taken from various instruments spanned the full range of dementia. Such items were categorized according to the earliest stage at which the raters judged them relevant.

The pool of 377 selected items was listed in rank-order according to the averaged ratings of the degree of severity. Within each set of tied rankings i.e., items with equal ratings of "severity" items were ordered according to their ratings for "clinical relevance" and "therapy sensitivity". This allowed the identification of the items that were judged most relevant and sensitive to change for each rating of severity.

Results and discussion

Both, the ranking of the 377 items according to stage of dementia and the content of the items served as a guide to select specific items representing impairments of ADL appropriate for the whole range of dementia i.e., from early "prodromal" to "extremely severe" dementia. In addition, a preliminary investigation based on clinical experience for staging the course of dementia was conducted. A scale of the type shown in Fig. 1 measuring the severity of dementia was provided for rating each of the 44 items. The 5 intervals indicated by descriptive adjectives ("Normal 70", "Prodromal", "Mild", "Moderate", "Severe" and "Extreme") were divided into two equal segments to provide 10 scoring intervals for rating each item.

Fourteen clinicians rated these 44 items according to the stage of dementia which seemed to be most appropriately described by the content of the item (see above mentioned procedure). Preliminary statistical analyses of these clinician ratings revealed the general usefulness of these items and that the items actually span the total range of the severity of dementia.

Ad (3). Another approach that was derived from the clinician rating approach was the construction of items based on diagnostic criteria and on items of the item pool appropriate for the assessment of mild and

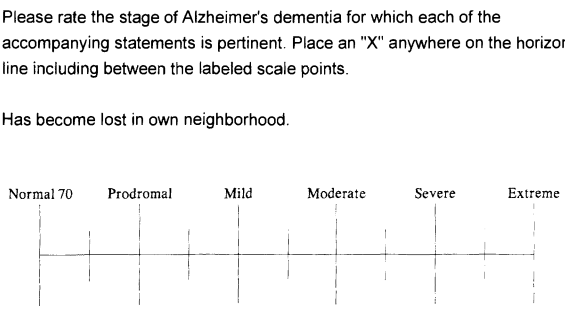

Fig. 1. Example of activity item

moderate dementia. The resulting scale consisted of a total of 65 rating scale items plus four open ended items which need to be completed by the rater.

One particular characteristic of this scale was that it described cognitive deficits causing impairments of activities of daily living. It was attempted to construct at least one item for each symptom of cognitive impairment listed in the descriptions of DSM-III-R or ICD 10 for the diagnosis of Alzheimer's disease or multi-infarct dementia.

The activities listed in this scale go beyond the ones usually mentioned under ADL or IADL (see Lawton and Brody, 1969). In short, the described activities can be categorized as that which White (1959) calls the effectance domaine. Activities of the effectance domaine are complex and higher level daily activities in the elderly population which are directed towards establishing and/or maintaining a control or mastery over the immediate environment (Sclan, 1993). Such activities are thought to be most vulnerable to dysfunction at the early stages of dementia (Kemp and Mitchell, 1992). The effectance domaine encompasses activities such as "skilled performance activities" (e.g., operating equipment, learning new tasks and procedures, hobbies, certain housekeeping tasks) and social roles (e.g., ability to relate to friends and family in a sensitive and rational manner). The total scale of 69 items assesses maintained competencies as well as functional deficits.

Ad (4). According to the recommendation of the steering committee (chair: B. Reisberg) the patient-based interview approach applied so-called focused interviews investigation impairments of performing ADL in daily life with patients and their collateral or caregiver. The patients needed to have the diagnosis of Alzheimer's disease or vascular dementia or mixed dementia according to ICD-10 (WHO, 1991). Patients with an additional psychiatric disorder or with other medical conditions that interfere with ADL were excluded. So far, 17 interviews have been analyzed. For this approach the following procedure for producing items was adapted:

(1) Summary of notes of clinical interviews
(2) Coding of clinical summary notes
(3) Restatement of coded segments
(4) Reduction of the wording of phrases and the grasp of common clusters (generalization)
(5) Item writing

As a first step the interviews were summarized; then the summaries were coded according to the activities of daily living mentioned. The subsequent steps resulted in higher levels of abstraction; the codes were restated and common wording of phrases or common clusters are extracted. Finally, items were formulated. The clusters were sorted by GDS stages (i.e., Global Deterioration Scale; Reisberg et al., 1982) and

Table 4. Suggested common clusters and possible items

GDS = 3
Leisure activities (reading):
– problems remembering story line and decreased reading Household chores and self-care
– porgets which household chores she needs to do
– porgets to take medication unless written down
GDS = 4
– Leisure activities (TV, VCR):
– inability and (or difficulty using remote control devices Self-care
– doesn't cut finger-nails or toe-nails
– cannot handle own medication even with written imstructions

common themes. Examples of common clusters are listed in Table 4. For GDS 3 and "Leisure Activities" one common theme was "Problems remembering story line and decreased reading". For GDS 3 and "Household Chores and Self-care" two themes most often mentioned were "Forgets which household chores she needs to do" and "Forget to take medication unless written down".

For GDS 4 and "Leisure Activities" the "Inability and/or difficulty using remote control devices" was frequently mentioned. For GDS 4 and "Self-care" two themes seem to be most promising for scale construction "Doesn't cut finger-nails or toe-nails" and "Cannot handle own medication even with written instructions".

Ad (5). For the expert-based interview approach two studies were conducted. In the first study experts were asked about the kinds of ADL problems faced by the patients on the basis of their experience. As a result clinicians had difficulty in identifying such problems and when problems were mentioned they tended to be quite consistent with those commonly found in rating scales e.g., Global Deterioration Scale (GDS). In a second study, the experts were presented with the 44 items derived from the total item pool of 92 scales and were asked to list the appropriate GDS stage for each item. Nine experienced (non-research) clinicians (5 Americans and 4 Germans) took part in this study. Intercultural differences were noted. For example the items "Often searches for misplaced eyeglasses or other object" and "Cannot use a city or highway map even if vision is not impaired" were rated as more appropriate for the indication of cognitive decline by American clinicians.

References

CPMP Working Party on Efficacy of Medicinal Products (1993) Note for Guidance. Antidementia Medicinal Products

Erzigkeit H (1989) Diskussionspapier: Entwicklung internationaler ADL-Skalen für den Einsatz bei Patienten mit dementiellen Erkrankungen (unpublished)

Erzigkeit H, Lehfeld H, Schultheiß O (1993) International dementia research: comments on the validation of psychometric measures and proposals for the definition of clinical relevance. In: Hindmarch I, Stonier PJ (eds) Human psychopharmacology: measures and methods, vol 4. Wiley, Chichester

Gutzmann H (1988) Senile Demenz vom Alzheimer-Typ. Enke, Stuttgart

Kanowski S, Ladurner G, Maurer K, Oswald WD, Stein U (1990) Empfehlungen zur Evaluierung der Wirksamkeit von Nootropika. Z Gerontopsychol Gerontopsychiatr 3:67–79

Katz S, Ford AB, Moskowitz RW, Jackson BA, Jaffee MW (1963) Studies of illness in the aged. The index of ADL: a standardized measure of biological and psychosocial function. J Am Med Assoc 185:94 ff

Kemp B, Mitchell J (1992) Functional assessment in geriatric mental health. In: Birren J, Sloane RB, Cohen G (eds) Handbook of mental health and aging, 2nd ed. Academic Press, New York, pp 671–697

Lawton MP, Brody EM (1969) Assessment of older people: self-maintaining and instrumental activities of daily living. Gerontologist 9:179–186

Methodenforum (1990) Technical report for the Psychiatry Department of the University of Erlangen-Nürnberg

Poon LW (1993) On the paradox of improving sensitivity of ADL scales for the detection of behavioral changes in early dementia. Sixth Congress of the International Psychogeriatric Association, September 5–10, 1993, Berlin (Abstract)

Reisberg B, Ferris SH, de Leon MJ Crook T (1982) The Global Deterioration Scale for assessment of primary degenerative dementia. Am J Psychiatry 139:1136–1139

Sclan SG (1993) Common protocol. A functional assessment instrument for pharmacologic trials in dementia. Working paper of the international ADL project (unpublished)

White RW (1959) Motivation reconsidered: the concept of competence. Psychol Rev 66:297–333

World Health Organization (1992) The ICD-10 classification of mental and behavioural disorders. WHO, Geneva

Correspondence: Dr. M. Stemmler, Psychiatrische Universitätsklinik, Schwabachanlage 6, D-91054 Erlangen, Federal Republic of Germany

Trial designs and outcome variables in anti-dementia drug testing

M. Panisset and **S. Gauthier**

McGill Centre for Studies in Aging, St-Mary's Hospital, Montreal, Quebec, Canada

Summary

Designing a protocol for a therapeutic trial in Alzheimer's disease (AD) is a very complex undertaking and is based on knowledge accumulated over the years about the manifestations of the disease and its diagnostic criteria, its natural history and its pathophysiology. Knowledge about the new compound from its early pre-clinical and clinical phases of development is equally important. Selection of validated outcome variables and a well coordinated project are the other keys for the successful and scientific validation of new medication for the treatment of AD.

Introduction

Alzheimer's disease (AD) cannot be considered only as a disease, but also as a societal concern. It has repercussions at different levels. Patients lose memory and other cognitive functions which makes it more difficult for them to relate to the world but also to themselves. Loss of autonomy ensues. This can be associated with depression especially in the initial stages of the disease, but usually gives way to an unawareness of their condition. Relative-caregivers may also suffer from witnessing helplessly the decline of a loved one, from the broken dream of an active retirement. The burden and the cost of taking care of AD patients grows heavier along with the progressive loss of the patients' autonomy. These costs are shared in various proportions between patients' family, third party payers and governments.

Physician services, hospital care, nursing home care, medications, adult day care, homemaker services, respite care, transportation services are the direct costs. whereas contribution and productivity loss associated to illness and premature death, unpaid informal caregiver services provided by family members and friends are the indirect costs.

The cost related to the care of AD patients in the US would be around $26–27 000 per year per patient and the total annual cost is estimated at 58 billion dollars (Huang et al., 1985). The figure of AD related expenses would not be complete if we would leave out the money invested into research and educational programs (Max, 1993). As the seniors represent the fastest growing segment of the population of industrialized countries and ten percent of them are afflicted by AD, these costs are expected to continue to rise.

With the limited success of most therapeutic attempts up to now in AD, new definitions for efficacy have become necessary. Patients exposed to a treatment need to show not only an improvement on neuropsychological testing, which could be an isolated "laboratory" finding, but also that this improvement is of significance in everyday life. For example, better performances on orientation tasks need to be associated to an increased ability to find one's way around in the neighbourhood. In the near future, another requirement may be added by governmental agencies (the Food and Drug Administration in the United States, for example) to measure the efficacy of a treatment and it has to do with decreasing the cost of care related to the disease. Indeed, the treatment that will produce a considerable improvement or a long lasting stabilization of cognitive functioning is likely to show a significant regain or stabilization of functional autonomy, and consecutively, will help reduce the costs.

In order to fulfil these requirements satisfactorily, the proper design of the research protocol and the appropriate choice of outcome variables are of paramount importance. Many factors, some relating to the disease and some to the nature of the medication, will influence the design of the protocol and need to be taken into account.

The disease: Alzheimer's disease

The accuracy of the diagnosis of AD has improved significantly over the recent past years, averaging a clinical pathological concordance of 85% (Lopez et al., 1990; Kukull et al., 1990). This has been made possible in part by the publication of specific criteria such as those of the Diagnostic and Statistical Manual of Mental Disorders, third edition revised (APA, 1987), and of the NINCDS/ADRDA (McKhann et al., 1984). Table 1 shows the DSM-III-R criteria and Table 2 shows suggested steps in making the diagnosis of AD.

A good history from the patient is often impossible and thus information obtained from a reliable informant is necessary for the diagnosis. The input from the informant will also be required during the study for the observation of changes in behaviour and in daily activities. The informant will make sure the patient is compliant with the treatment schedule and the evaluation visits.

Table 1. DSM-III-R criteria for dementia (modified from Forette et al., 1992)

A. Impairment in short and long-term memory
B. Impairment in at least one other cognitive function such as abstract thinking, impaired judgment, aphasia, apraxia, agnosia, constructional difficulties, and personality changes
C. The disturbances in A and B significantly interferes with work or usual social activities or relationships with others
D. Does not occur only during delirium
E. The disturbance cannot be accounted for by any non-organic mental disorder

Table 2. Suggested steps for the diagnosis of Alzheimer's disease (modified from Forette et al., 1992)

1. History and physical examination
2. DSM-III-R criteria for dementia
3. Evaluation of the severity of dementia with the MMS
4. Laboratory tests:
– complete blood count
– thyroid function tests
– serum electrolytes and glucose
– urea and creatinine
– vitamin B 12 and folate
– VDRL
5. Cerebral computed tomography

The differentiation between early AD and the manifestations of normal aging may be difficult. A follow-up visit at least six months later may at times clarify the issue (Huff et al., 1987). Usually, protocols will be targeted to patients with a mild to moderate degree of dementia. These patients should not show a ceiling effect on the cognitive measures in order to be able to later show an improvement. A maximum Mini-Mental State examination (MMS, Folstein et al., 1975) score of 24 to 26 is often used for this purpose.

Sometimes the presentation of a depressed patient may be confusing. Depression scales are used to help but should not replace the clinician's impression. Nevertheless, since depression can interfere with performance, it is wise to exclude patients with significant depressive symptoms. Though it may not be the most appropriate scale to be used in the demented elderly because of the many questions pertaining to symptoms common to both depression and dementia, a Hamilton Depression scale (Hamilton, 1967) score of more 13 over 27 points usually represents an exclusion criteria.

Differentiation between AD and Vascular Dementia (VaD) may also prove difficult. Scales such as the ischemic score (Hachinski et al., 1975) or any of its numerous versions are used to help in this task (Gräsel et

al., 1990; Loeb and Gandolfo, 1983; Rosen et al., 1980), but as we know more about VaD and its relationship to AD, using such scales may be "oversimplistic" (Fischer et al., 1991; Lopez et al., 1993; Parlato et al., 1993). The NINDS-AIREN criteria (Roman et al., 1993) for VaD are a step further in delineating the border between these disorders.

Laboratory tests (including a complete blood count, electrolytes, glycaemia, urea, creatinine, liver and thyroid tests, a VDRL, dosage of vitamin B 12 and folate), a cerebral computed tomography scan (CT) and an electroencephalogram (EEG) will help eliminate concomitant diseases which could present with dementia (Panisset, 1993). Some of these tests will also be used as a baseline in order to detect any laboratory changes that could be related to the medication.

Some medications used by patients for unrelated conditions may have central nervous system effects and may interfere with the evaluation of the cognitive functioning at baseline and during the study.

For obvious reason, patients who suffer from life threatening disease should also be excluded from protocols.

The severity of the dementia is usually measured with the MMS or with the Global Dementia Scale (Reisberg et al., 1982), or more recently with the Clinical Dementia Rating (CDR, Berg, 1988; Morris, 1993). Patients with more severe disease have more widespread cellular losses so treatment strategies aimed at the preservation of surviving neurons are not realistic.

Neurotransmitter precursors may also not be as effective in advanced stages, though this is still not clearly demonstrated.

At the other end of the spectrum and as mentioned previously, patients with mild dementia or previous high levels of intellectual functioning may show a ceiling effect on cognitive measures used as outcome variable.

Apart from the accuracy of the diagnosis of AD and the severity of the dementia, more and more attention is given to the different syndromes of AD. Some patients present predominantly verbal deficits and some others more visuo-spatial problems; some present a frontal behaviour that makes it difficult to differentiate from frontal lobe dementia, Pick's disease, etc. Each of these syndromes may not be caused by AD pathology as has been shown recently in the case of primary progressive aphasia (Mesulam, 1992). Moreover, AD patients with predominant verbal impairments may have a faster cognitive decline (Boller et al., 1991).

Whether age at onset is important in the clinical manifestations of the disease has been controversial, but it seems to be agreed upon that biochemical differences exists (Rossor et al., 1984; Francis et al., 1985). Younger patients would have more profound neurotransmitter deficits, whereas older ones would have a biochemical profile similar to that of their age matched controls.

Other sources of variation in the rate of progression of AD include familial history of AD, presence of psychotic features, extra-

pyramidal signs and myoclonus (Mayeux et al., 1985; Chui et al., 1985).

The selection of the population is of course of major importance. A high degree of homogeneity may help in the physiopathological interpretation of the results, but advocates for a looser screen emphasize that effective treatments will be marketed for AD patients with concomitant disorders and different degrees of severity. As new treatments continue to progress in their evaluation, entry criteria should include more of these patients.

The nature of the medication

Since the cause of AD is still unknown, most treatments have been symptomatic. New medications are being tried that would slow down the progression of the disease. As our understanding of the etiology(ies) and risk factors increases, we should witness the emergence of therapies to prevent the disease or delay its onset in people identified as being at high risk (Poirier et al., 1993).

The purported action of a specific agent calls for a specific protocol design. Trials with symptomatic purpose should last a relatively short time. It may even be difficult to prolong the trial for more than three months if the compound is particularly promising.

An agent aimed at slowing the progression of the disease will need early stage probable AD patients. The duration of the study should be of at least one year (Growdon, 1993). A proper wash out period must be planned to differentiate preventive from symptomatic effects as we learned from the DATATOP experience (The Parkinson Study Group, 1993).

Experience with the new treatment is obtained through a series of studies on the animal and on the human. In man, the protocols are classified as phase I, II and III (Table 3). Phase IV takes place when the

Table 3. Definitions of therapeutic trial phases

Phase 1 (clinical pharmacology)
– initial introduction of the treatment into humans
– usually normal volunteer subjects
– determination of the levels of toxicity
– ± determination of pharmacologic effect may be followed by early dose ranging studies in patients for safety ± evidence of effectiveness
Phase II (clinical investigation)
– controlled clinical trial
– determination of effectiveness and relative safety
– closely monitored patients (< 200)
Phase III (clinical trials)
– expanded controlled and uncontrolled trials
– additional evidence of effectiveness for specific indications
– more precise definition of drug related adverse effects

medication has been marketed and is aimed at gaining more information and developing new indications.

In the early phases of testing, the main question will be whether the medication has any harmful effect or is toxic. Measures of attentional processes should be implemented early in the evaluation of a new compound in order to detect the presence of a confusional state induced by the medication. This is of particular importance in studies aimed at improving memory and directed at the elderly population which is so sensitive to medications side effects.

These early phases will provide notions about the pharmacology of the drug and empirical evidence of efficacy in AD. Data on maximal tolerated doses, on adverse effects will also be provided. A number of issues can be dealt within these studies that will have important consequences for future and larger clinical trials (Gauthier et al., 1990), for example, whether the drug is associated with withdrawal effects. That has been the case in the American Tacrine study (Davis et al., 1992), which used a cross-over design, where patients exposed to the drug in the first part of the study were switched after a short wash out period to placebo in the second part, and vice and versa. A significant carry-over effect was noted between the two parts of the study and a possible withdrawal effect rendered the results difficult to interpret.

The different routes of administration have their respective problems. Intravenous injections may carry a risk of phlebitis that is not balanced by the chance of some benefit from the medication tested. This ethical question is of even greater importance in more aggressive approaches such as the intrathecal injections. In medications given orally, compliance must be enforced and assessed with the help of an informant.

The research protocol

Study design

Parallel design is the most appropriate when little is known about the drug in particular the pharmacokinetic parameters, possible existence of active metabolites, etc. Also it has been demonstrated, especially in AD, that patients subjected to placebo are capable of both subjective and objective improvements (Ellenberg, 1990). The placebo-response effect may be even more apparent to family members of the patients who are desperate for an effective treatment which can influence some of the outcome variables.

Once the first medication recognized as anti-dementia properties will be marketed, we may be faced with comparing groups of patients on this marketed medication and the tested drug. Analyses of efficacy and toxicology will then be much more complex.

The progressive nature of AD has great importance for drug trials. As we learned in the Canadian Tacrine trial (Gauthier et al., 1990), patients cannot be used as their own control in a cross-over design because they may not be back to their previous level of functioning a few months later. Also the information that a particular compound is capable of causing a carry-over effect is often missing at the time of clinical testing.

A wash-out phase is useful in determining the symptomatic and preventive effect in drugs where both these effects are possible such as with selegiline in Parkinson's disease (The Parkinson Study Group, 1993).

Blind and randomization

Trials need to be blinded so that the results will be minimally biased. Patients, treating physicians and evaluators of the efficacy and of the toxicity of the new treatment need to be blinded. In instances where the blind could be breached either because the compound may show side effects or because the compound is administered at maximal tolerated doses (making the event of side effects more likely), evaluation of toxicity and of efficacy need to be performed by different and independent clinicians.

Each protocol needs to be individually examined in order to detect any factor that could brake the blind. The community has become so concerned about blind that some trials are conducted with the monitoring committee and the statisticians performing interim analyses blinded to the treatment assignment.

Randomization

Randomization is necessary to avoid the possibility of bias in assignment of subjects to particular treatment arms, to end up with groups that are balanced with regard to factors that may affect the outcome of treatment (whether these factors are known or unknown), and to provide validity to the statistical tests of significance that will be used in the comparative analyses (Ellenberg, 1990).

The enrichment protocol was developed in the French and in the American Tacrine trials (Davis et al., 1992). It consists in selecting only those patients who show a positive symptomatic response to the medication for the later double blind part of the study. This method has the advantage of requiring a smaller number of subjects to show a treatment effect, but responders to a first exposure may not show the same response on subsequent ones (Gauthier and Gauthier, 1993).

Outcome variables

For statistical reason, efficacy of a new treatment needs to be measured with a small number of outcome variables (primary). One out of 20 variables is expected to show significant change only by chance. These outcome variables are usually dictated or strongly recommended by the regulatory agencies. At this time, the Food and Drug Administration of the United States requires evidence of improvement both on a relevant objective measure of cognitive performance and on an independent clinically ascertained global impression of change. The new treatment needs to show ecological cognitive improvements. The improvement in intellectual functions as shown on the neuropsychological tests need to be of significance in everyday life to the patients and their families and not to be restricted only to abstract results of neuropsychological tests. Furthermore, because of the economical situation affecting health care systems of many countries, a drug may also need to be pharmacoeconomically advantageous, i.e. it can delay the need for or the amount of services.

Secondary outcome measures may not be used primarily to assess the efficacy but can help better define the effects of the tested medication or to further our knowledge on the natural evolution of the disease by using data on the placebo group.

Outcome measures need to respond to specific requirements in order to be used in clinical trials. Since the same measures will be used at different intervals, they need to show a good test-retest reliability profile. Learning and familiarization effects between administrations of the test was thought to be unlikely in AD but was recently shown especially when tests have a large speed component, require an unfamiliar or infrequently practiced mode of response or have a single solution (Forette et al., 1992) and particularly if it is easily conceptualized once it is attained (Galasko et al., 1993). A learning effect was even shown in the American Tacrine study (Davis et al., 1992). Options to control these effects include using different equivalent versions of the same test, obtaining the maximal performance at baseline by repetitive administrations of the same test or using a cross-over placebo controlled design.

The measures also need to show a high inter-rater reliability since different raters from different centres and sometimes from different countries will be involved in the same study. Training sessions are usually provided before a study starts in a way to ensure homogeneity in the testing techniques.

The tests must have a range of scores that enables better performers to have higher scores without showing a ceiling effect and that allows a downward scoring to follow patients during the evolution of the disease. In the French Tacrine trial the Alzheimer's Disease Assessment Scale (ADAS, Rosen et al., 1984), which is the actual standard cognitive battery in therepeutic trials in the United States and Canada, has been

shown to have a ceiling effect in patients of higher education, which could have prevented the study from showing any benefit.

Adequate cognitive measures for patients in advanced stages of dementia such as the Severe Impairment Battery that we have developed (Panisset et al., 1994) are now available. This will be of major importance when the drugs are extended to these severely impaired populations.

Tests need to be as objective as possible and quantifiable. Subjective outcome measures need a much larger number of participants to gain the same validity as objective measures, and human judgment even then would still be a possible bias. If subjective assessment cannot be avoided, multiple assessment by independent evaluators may be necessary to reduce variability and increase validity.

Cognitive tests need to target functions thought to be improved by the new treatment. In AD this is usually memory, but since memory comprises many specific functions that are subserved by different anatomical and neurotransmitter systems, tests need to be able to assess these various components (learning, free and cued recall, recognition, working memory, associative, implicit versus explicit, verbal versus visuo-spatial, short-term versus long-term memory).

Mood state may influence the cognitive functioning and thus needs assessment before each testing.

Activities of daily living (ADL) may need to be divided into instrumental (telephone, shopping, housework, taking medications) and physical (eating, dressing, washing) since they are not affected to the same degree and at the same time in the disease evolution.

Monitoring of the clinical trial

Information derived from preclinical and early clinical phase studies help in deciding the allowed concomitant medications, the necessary safety measures (adverse effects and laboratory changes), etc. Regulations concerning premature terminations and reasons to break the blind all need to be clearly defined. Well kept data entry forms and regular visits as scheduled are necessary for the successful analysis of the study results. Independent data safety and monitoring committees for the welfare of the patients need to be put in place to assess unexpected toxicity. An interim analysis in long lasting trials may show early definite evidence of efficacy and warrant an early termination of the trial.

Ethical considerations

In placebo-controlled studies the physician and the family may feel uneasy to take the chance not to get the active treatment. This is

particularly true in AD where even if there is no fully effective treatment, treatment provides hope for the caregivers. One possible solution would be to use historical control groups, but both groups would no have been tested under the same conditions. Biases with non-concurrent groups include the possibility that patients characteristics will not be similar and comparable, the method of selection may differ, the diagnostic criteria may have changed over time. It has been well shown that poorly controlled studies give results that are more dependant on the method of selection that on the therapy under study (Ellenberg, 1990).

Taking the risk of being on placebo must be weighed against the need to complete a valid study that allow us to definitely assess both the efficacy and the toxicity of an untested treatment for all future patients. If proved efficacious, all future patients will benefit from the drug and will not suffer from unnecessary toxicity or submission to the cost and inappropriate treatment with a worthless therapy (Ellenberg, 1990). Furthermore, patients are usually given the chance to receive the medication at the end of the trial, at least until the final results of the study are known.

Conclusion

Designing a protocol for a therapeutic trial in AD is a very complex undertaking and is based on knowledge accumulated over the years about the manifestations of the disease and its diagnostic criteria, its natural history and its pathophysiology. Knowledge about the new compound from its early pre-clinical and clinical phases of development is equally important. Selection of validated outcome variables and a well coordinated project are the other keys for the successful and scientific validation of new medication for the treatment of AD.

Acknowledgments

The preparation of this chapter was supported in part by a grant from the Medical Research Council of Canada/The Canadian Alzheimer Society/Price Daxion (Dr. Panisset).

References

American Psychiatric Association (1987) Diagnostic and statistical manual of mental disorders, 3rd edn revised. American Psychiatric Association, Washington DC, pp 97–164

Berg L (1988) Clinical dementia rating (CDR). Psychopharmacol Bull 24:637–639

Boller F, Becker JT, Holland A, Forbes M, Hood PC, McGonigle-Gibson KL (1991) Predictors of decline in Alzheimer's disease. Cortex 27:9–17

Chui HC, Lee Teng E, Henderson VW, Moy AC (1985) Clinical subtypes of dementia of the Alzheimer type. Neurology 35:1544–1550

Davis KL, Thal LJ, Gamzu ER, et al (1992) A double blind, placebo-controlled multicenter study of Tacrine for Alzheimer's disease. NEJM 327:1253–1259

Ellenberg JH (1990) Clinical trials. In: Theodore WH (ed) Clinical neuropharmacology, neurologic clinics. WB Saunders, Philadelphia, pp 15–30

Fischer P, Jellinger K, Gatterer G, Danielczyk W (1991) Prospective neuropathological validation of Hachinski's Ischaemic Score in dementias. J Neurol Neurosurg Psychiatry 54:580–583

Folstein MF, Folstein SE, McHugh PR (1975) "Mini Mental State": a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189–198

Forette, F, Panisset M, Boller F (1992) Clinical trials in cognitive impairment in the elderly. Aging Clin Exp Res 4:239–250

Francis PT, Palmer AM, Sims NR, Bowen DM, Davison AN, Esiri MM, Neary D, Snowden JS, Wilcock GK (1985) Neurochemical studies of early-onset Alzheimer's disease. Possible influence on treatment. NEJM 313:7–11

Galasko D, Abramson I, Corey-Bloom J, Thal LJ (1993) Repeated exposure to the Mini-Mental State Examination and the Information-Memory-Concentration Test results in a proactive effect in Alzheimer's disease. Neurology 43:1559–1563

Gauthier S, Bouchard R, Lamontagne A, Bailey P, Bergman H, Ratner J, Tesfaye Y, Saint-Martin M, Bacher Y, Carrier L, Charbonneau R, Clarfield M, Collier M, Dastoor D, Gauthier L, Germain M, Kissel C, Krieger M, Kushnir S, Masson H, Morin J, Nair V, Neirink L, Suissa S (1990) Tetraaminoacridine-lecithin combination treatment in patients with intermediate-stage Alzheimer's disease. Results of a Canadian double-blind, crossover, multicenter study. NEJM 322:1272–1276

Gauthier S, Gauthier L (1993) What we have learned from the THA trials to facilitate testing of new AChE inhibitors. Prog Brain Res 98:527–429

Gräsel E, Cameron S, Lehrl S (1990) What contribution can the Hachinski ischemic scale make to the differential diagnosis between multiinfarct dementia and primary degenerative dementia? Arch Gerontol Geriatr 11:63–75

Growdon JH (1993) Tacrine in Alzheimer's disease. NEJM 328:810

Hamilton M (1967) Development of a rating scale for primary depressive illness. Br J Soc Clin Psychol 6:278–295

Hachinski VC, Iliff LD, Zilhka E, Du Boulay GH, McAllister VL, Marshall J, Ross Russell RW, Symon L (1975) Cerebral blood flow in dementia. Arch Neurol 32:632–637

Huang L, Cartwright W, Hu T (1985) The economic cost of senile dementia in the United States. Public Health Rep 1988/103:3–7

Huff FJ, Becker JT, Belle SH, Nebes RD, Holland AL, Boller F (1987) Cognitive deficits and clinical diagnosis of Alzheimer's disease. Neurology 37:1119–1124

Kukull WA, Larson EB, Reifler BV, Lampe TH, Yerby M, Hughes J (1990) Interrater reliability of Alzheimer's disease diagnosis. Neurology 40:257–260

Loeb C, Gandolfo C (1983) Diagnostic evaluation of degenerative and vascular dementia. Stroke 14(3):399–401

Lopez OL, Swihart AA, Becker JT, Reinmuth OM, Reynolds III CF, Rezek DL, Daly III FL (1990) Reliability of NINCDS-ADRDA clinical criteria for the diagnosis of Alzheimer's disease. Neurology 40:1517–1522

Lopez OL, Becker JT, Rezek D, Wess J, Boller F, Reynolds III C F, Panisset M (1992) Neuropsychiatric correlates of cerebral white-matter radiolucencies in probable Alzheimer's disease. Arch Neurol 49:828–834

Lopez OL, Larumbe MR, Becker JT, Rezek D, Rosen J, Klunk W, DeKosky ST (1994) Reliability of NINDS-AIREN clinical criteria for the diagnosis of vascular dementia. Neurology 44:1240–1245

Max W (1992) The economic impact of Alzheimer's disease. Neurology 43 [Suppl 4]:S6–S10

Mayeux R, Stern Y, Spanton S (1985) Heterogeneity in dementia of Alzheimer type: evidence of subgroups. Neurology 35:453–461

McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of the Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology 34:939–944

Mesulam MM, Weintraub S (1992) Primary Progressive Aphasia: sharpening the focus on a clinical syndrome. In: Boller F, Forette F, Khachaturian Z, Poncet M, Christen Y (eds), Heterogeneity of Alzheimer's disease. Springer, Berlin Heidelberg New York Tokyo, pp 43–66

Morris J (1993) Clinical Dementia Rating (CDR): current version and scoring rules. Neurology 43:2412–2414

Panisset M (1993) How to detect and confirm dementia. Med N Am 6:490–493

Panisset M, Roudier M, Saxton J, Boller F (1994) Severe Impairment Battery: a neuropsychological battery for severely demented patients. Arch Neurol 51:41–45

Parlato V, Panisset M, Lopez OL, Becker JT, Dalla Barba G, Bonavita V, Boller F (1993) A longitudinal study of memory deficits in probable Alzheimer's disease and mixed dementia. J Clin Exp Neuropsychol 15:30–31

Poirier J, Davignon J, Bouthillier D, Kogan S, Bertrand P, Gauthier S (1993) Apolipoprotein E polymorphism and Alzheimer's disease. Lancet 342:697–699

Reisberg B, Ferris SH, de Leon MJ, Crook T (1982) The Global Deterioration Scale (GDS): an instrument for the assessment of primary degenerative dementia (PDD). Am J Psychiatry 139:1136–1139

Román GC, Tatemichi TK, Erkinjuntti T, Cummings JL, Masdeu JC, Garcia JH, Amaducci L, Orgogozo J-M, Brun A, Hofman A, Moody DM, O'Brien MD, Yamaguchi T, Grafman J, Drayer BP, Bennett DA, Fisher M, Ogata J, Kokmen E, Bermejo F, Wolf PA, Gorelick PB, Bick KL, Pajeau AK, Bell MA, DeCarli C, Culebras A, Korczyn AD, Bogousslavsky J, Hartmann A, Scheinberg P (1993) Vascular dementia: diagnostic criteria for research studies. Neurology 43:250–260

Rosen WG, Terry RD, Fuld PA, Katzman R, Peck A (1980) Pathological verification of ischemic score in differentiation of dementias. Ann Neurol 7:486–488

Rosen WG, Mohs RC, Davis KL (1984) A new rating scale for Alzheimer's disease. Am J Psychiatry 141:1356–1364

Rossor MN, Iversen LL, Reynolds GP, Mountjoy CQ, Roth M (1984) Neurochmical characteristics of early and late onset types of Alzheimer's disease. Br Med J 288:961–964

The Parkinson Study Group (1993) Effects of tocopherol and Deprenyl on the progression of disability in early Parkinson's disease. NEJM 328:176–183

Correspondence: M. Panisset, M.D., McGill Centre for Studies in Aging, St. Mary's Hospital, 3830 Lacombe Avenue, Montreal, Quebec, H3T 1M5 Canada

Cognitive deterioration in old age and in the course of dementia

W.D. Oswald and **K. Tritt**

Institute of Psychology, University of Erlangen-Nürnberg,
Federal Republic of Germany

Summary

Cognitive deterioration in old age in the course of dementia can be studied by a variety of psychometric assessment scales, the recent developments of which are briefly summarized. They concern performance, fluid functions, memory performance, sensoric memory and other items that show different changes in normal and pathological aging. In dementia, progressive deterioration of crystallized functions contribute to the patient's loss in quality of life. In demented individuals, difficulties in processing, retrieval and speed are predominant. In a series of 200 patients with Alzheimer disease and multi-infarct dementia, no significant differences with regard to various basic cognitive functions were found and, from the psychometric point of view, these two types cannot be distinguished. Diagnosis of dementia can be substantiated by the use of recently developed highly sensitive instruments that may substantiate the suspected clinical diagnosis of organic brain syndromes.

Introduction

Until recently, it was commonly assumed that when examining cognitive changes in the elderly and illnesses related to dementia, one should mainly expect deficiencies. Due to recent findings of fundamental research in psychogerontology, such a simplified view can hardly be adhered to any longer. Up to this day, an other frequent notion is that the memory of demented patients is impaired in its entirety. This view also requires some revision, since different functions are impaired in various degrees at differing points within the course of illness.

Ageing is accompanied by a variety of morphological and biochemical changes. As long as the deterioration does not fall short of an assumed (Meier-Ruge, 1983) reserve capacity of the central nervous system (see Fig. 1), these changes alone do not compromise an illness,

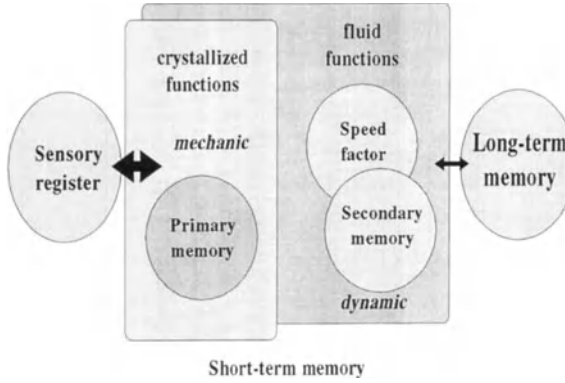

Fig. 1. Basic dimensions of the memory (according to Oswald and Fleischmann, 1992)

as understood in the usual sense. Clinical syndromes, like senile dementia of the Alzheimer's type (SDAT) or multi- infarct dementia (MID), first become manifest, when this occurs. It can therefore be assumed today that the absence of or merely minor differences can be observed between patients with beginning dementia and healthy elderly on the level of psychometrically assessable cognitive performance. Significant differences are first noted later in the course of illness; initially these differences can only be distinguished quantitatively, while qualitative differences can first be discerned in the late stages. For the sake of clarification, before discussing specific aspects of dementia and questions pertaining to its diagnosis, a brief general presentation on the latest research status will be given.

Dedifferentiation of psychic functioning

Research has shown that a dedifferention of psychic functioning (Oswald and Gunzelmann, 1991) occurs with increasing age and increasing progression of dementia: While for adolescents, performance on individual functions (e.g., verbal and numerical abilities) varies independently (an individual can achieve good scores in one and poor results in other areas). With increasing age, a dedifferention towards only two independent dimensions can be found. Following Cattell (1971) these two independent dimensions are commonly referred to as crystallized and fluid functions (Fleischmann and Oswald, 1982).

Performance that is independent of cognitive speed and highly dependent on practise and education, involving for instance knowledge of language as well as cultural knowledge, has been designated as crystallized functions. Reasoning and learning poems by heart can be viewed as examples of such abilities.

Fluid functions, in contrast, are conceived of as the content independent, basic cognitive functions that enable a flexible assimiliation and processing of information. Since these abilities are composed of functions dependent on rapid performance, they are also frequently referred to as speed performances or speed of information processing. The intellectual and knowledge-related requirements are rather marginal. It is not only important to achieve a right answer, but even more relevant to achieve it quickly. That these performances are less dependent on milieu and educational factors but mainly genetically determined has been shown for instance through the comparision of mono- and dizygotic twins (Lienert et al., 1981). Processing a concentration test or crossing the street at a crosswalk can serve as examples of fluid functions.

Defining separate partial functions has also proven itself efficient on the area of memory performance. Next to long-term memory – three types of short-term memory are differentiated lately: sensoric memory, primary short-term and secondary short-term memory (Fig. 1).

The term sensoric memory has been applied to such short-term storaging processes that save impressions from the environment up to a few seconds and transfer them to primary short-term memory. The major task of sensoric memory is a filtering function. Sensoric memory focuses our attention and enables information to progress to the primary short-term memory and as such to our consciousness.

Since all information is mechanically held present for a short period of time (up to a span of a few minutes), primary short-term memory is a prerequisite of our conscious experiencing. Comparable to learning by heart, the internal reproduction simultaneously facilitates a mechanical embedment and linkage to information already stored in long-term

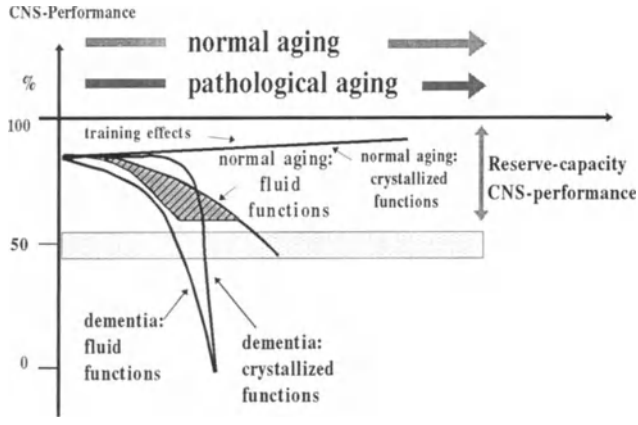

Fig. 2. Cognitive performance in normal and pathological brain ageing (Oswald and Fleischmann, 1992)

memory. Whereas primary short-term memory can be viewed more as a mechanical procedure, the term secondary short-term memory serves to designate all those dynamic and active operations that process the arriving information and achieve the information's permanent embedment in the already existing knowledge structure of permanent memory.

Age-correlated changes

Viewed in the light of this model, the following findings pertaining to the normal process of ageing have been established (cf., Fig. 2):

Crystallized functions are not subjected to age-correlated deterioration. These functions, generally regarded as the manifestation of knowledge accumulated over the biographical lifespan, can be maintained or even improved in the later age phases. The ability to learn poems by heart, for instance, can be maintained in old age. To a great extent, crystallized cognitive performance reflects a manifestation of accumulated experience and learning.

In contrast, fluid or power performance is affected by progressive decline, which already starts at the age of thirty. This finding implies that the more an individual's life-age has advanced, the more time the individual's processes of cognition and action require. One can no longer simultaneously process the same amount of information at an appropriate speed. The decline of "fluid" functions is assumed to accompany biological processes of deterioration and, as such, biological ageing.

In relation to the described memory processes, it should be noted that processes of sensoric as well as of secondary short-term memory are correlated with the already discussed speed dependent, basic fluid cognitive functions. This relationship becomes increasingly closer with an increase of age (Fleischmann, 1992) (cf., Fig. 2). Especially those functions of memory are impaired that pertain to active assimilation and more enduring imprinting of new information. Passive and only short-term retention is, on the contrary, hardly reduced in old age.

In the course of ageing, storing processes indicate deficiencies in the sensoric register. In addition, the speed of assimilation, encoding and retrieval is reduced, thus complicating encoding as well as retrieval of information from long-term memory. These functions in turn are highly correlated with fluid and speed dependent performance, whereas no age-correlated deficits have been observed for the more mechanic abilities of memory (primary short-term memory) (Fleischmann, 1985). The following points are put forward as examples:

- No deficits are observed by mechanical memorizing by heart.
- Salient deficits have been found by the acquisition of knowledge on current affairs.

Today a more differentiated approach to the description of cognitive ageing is being taken: Cognitive ageing can be viewed as the general reduction of speed in the cognitive processes – especially of the speed in central information processing (Birren et al., 1979; Bashore et al., 1989; Salzhouse, 1985). This process also results in a retardation of acting and consequently in a reduction of everyday competences (Oswald, 1982).

Progression of dementia

In essence, the aforementioned results hold for normal as well as for pathological ageing of the brain. Recent research presumes that – in regard to the psychometrically assessable basic functions – brain organic syndromes (e.g., SDAT or MID) and the normal ageing process of the brain reveal to a great extent a parallel development (cf., Fig. 1). It can therefore be concluded:

1. *Crystallized functions:* As in the normal range, no clinically relevant differences could be detected when comparing elderly with mild to moderate stages of dementia to younger individuals or to healthy elderly. As in the case of healthy elderly, it has concurrently been shown that performance of demented patients can be improved in these areas through exercise and training. Contemporary intervention programs stress the systematic learning of techniques to compensate impairments in the area of basic competences. These impairments deteriorate progressively in the course of illness and contribute essentially to the patient's loss in quality of life.
2. *Fluid functions:* For the fluid functions of information processing as well as for those processes highly correlated to them and pertaining to sensoric and dynamic secondary short-term memory, apparent differences have been detected, which become even more pronouced the farther the dementing deterioration has progressed. In the case of pathological changes though, the described ageing development of fluid cognitive performance occurs earlier and progresses more rapidly than in healthy elderly. Comparing individuals suffering from moderate dementia [GDS 4 on the Reisberg-Scale (Reisberg et al., 1982)] to a representative control group, differences of up to three standard deviations have been reported on speed of information processing, as a key parameter of the fluid functions. For the crystallized functions (e.g., repeating numbers stored in the area of mechanical short-term memory), no clinically relevant differences have been observed.

The following memory performance impairments can be presumed for demented individuals (Fleischmann, 1992):

1. *Processing:* difficulties in retention of new information, due to impaired encoding, linking and memorizing of new contents (newspaper articles, announcements, names . . .).
2. *Retrieval:* impaired memory search processes resulting in more difficult access to contents (it's on the tip of my tongue . . .).
3. *Speed:* Reduced speed of assimilation, processing and searching in the memory (difficulties with fast or rapidly changing presentations of information, e.g., announcements from loudspeakers or crossing a street . . .).

Active information processing constitutes the focal point of these three areas, which should all be ascribed to the fluid functions. On these variables, the differences between normally ageing persons and demented persons can reach up to two standard deviations. These differences are much more distinct when comparing younger age groups. This finding reflects the clinical experience that dementia occuring in the middle aged is accompanyied by especially obvious cognitive decline.

The division of the described basic functional areas has also proven itself in this field of application. It should be restrictively noted that with further progession of the illness, an increasing fusion of both basic functions should be expected. In general though, the accompanying symptoms first become manifest in the later and more severe stages of senile dementia and MID. This development implies that the heretofore relatively unimpaired crystallized functions are also subjected to considerable deterioration and as such can no longer be used to compensate for and to stabilize basic competences.

SDAT versus MID

The reader might be surprised that dementia of the type SDAT and of the MID type has not been differentiated – even though they have distinct pathogenetic origins. Few studies pertaining to the test-psychological differentiation of SDAT and MID have been published so far (Perez et al., 1976; Wagner et al., 1987). In a recently published study, Fleischmann et al. (1991) conducted basic research on this topic, which was carried out on a sample of 200 carefully diagnosed SDAT and MID patients. The authors conclude that, with regard to the various basic cognitive functions, no significant differences could be found between both types of dementia. Regarding their cognitive deterioration, SDAT and MID patients can therefore be considered as being comparable.

Diagnosis of dementia

As shown above, dementia can not be equated with a common loss of functions. Different abilities change in diverse ways. The advent of

modern psychopathometrics, with its newly developed and highly sensitive instruments – made especially for the early detection of organic brain syndroms (including dementing processes, like SDAT and MID), takes these results under consideration in a manner directed by theory. Especially the Nuremberger-Ageing-Inventory (NAT) (Oswald and Fleischmann, 1992) should be appended to the list of empirically founded and highly sensitive devices. In the mean time, different parts of the NAI are available in English, German, French, Spanish, Swedish, Dutch, and Czech. The Zahlen-Verbindungs-Test (number combining test) "ZVT-G", a modification of the Trail- Making-Test, is a frequently used and scientifically highly respected test for the diagnosis of dementia (Oswald, 1986, 1990; Oswald and Roth, 1987; Reitan, 1958). The fluid function "speed of information processing" is assessed with the ZVT-G.

An interesting and promising new approach for an early and economic detection of organic brain syndromes focuses on the subjective experiencing of ageing: The Nürnberger-Selbsteinschätzungs-Liste NSL (Oswald et al., 1990) was developed as a screening instrument, especially made for application by general practitioners. The questionnaire, consisting of 20 items, is filled out by the patient. The NSL is easy to score and, through comparision of the sum-score, a suspected diagnosis of organic brain syndrome can be clarified with a high degree of probability.

This new questionnaire shows only slightly poorer results in regard to its efficiency in the diagnosis of cerebrale performance deficiencies and dementing processes, when compared to psychometric tests, like e.g. the Zahlen-Verbindungs-Test ZVT-G. However, in view of its practicability, this instrument has obvious advantages, due to its simplicity, easy administration and scoring. Hence, the Nürnberger-Selbsteinschätzungs-Liste NSL presents itself as an easy to administer and nevertheless highly sensitive diagnostic tool – and therefore as an interesting alternative to existing diagnostic approaches to the assessment of dementia.

References

Birren JE, Woods AM, Williams MV (1979) Speed of behavior as an indicator of age changes and the integrity of the nervous system. In: Hoffmeister F, Müller C (eds) Brain function in old age. Evaluation of changes and disorders. Springer, Berlin Heidelberg New York, pp 10–44

Bashore TR, Osman A, Heffley EF (1989) Mental slowing in elderly persons: a cognitive psychophysiological analysis. Psychol Aging 4:235–244

Cattell RB (1971) Abilities: their structure, growth, and action. Houghton, Boston

Fleischmann UM (1985) Gedächtnisbezogene Förderung im hohen Lebensalter. Rehabilitation 24:36–38

Fleischmann UM (1989) Gedächtnis im Alter. Huber, Bern
Fleischmann UM (1992) Das alternde Gedächtnis, Teil 1. Neue Forschungsergebnisse zur normalen und pathologischen Gedächtnisalterung. Allgemeinarzt (in press)
Fleischmann UM, Oswald WD (1982) Zum Konzept der flüssigen Intelligenz im hohen Lebensalter. In: Henning HJ (ed) Applications of linear structural relationships, models in social sciences: results from LISREL-V Workshop, May 3–7. Brem Beitr Psychol 17:11–35
Fleischmann UM, Oswald WD, Kanowski S, Deutsch H (1991) Senile Demenz vom Typ Alzheimer und Multi-Infarkt-Demenz – Eine explorative Analyse zur psychometrischen Differentialdiagnostik. Z Gerontopsychol Gerontopsychiatr 4:143–160
Lienert GA, Gebert A, Haimerl I (1981) Ist die Zwillingsforschung noch zeitgemäss? In: Michaelis W (Hrsg) Bericht über den 32. Kongress der Deutschen Gesellschaft für Psychologie in Zürich 1989. Hogrefe, Göttingen, pp 488–489
Meier-Ruge W (Hrsg) (1983) Der ältere Patient in der Allgemeinpraxis. Karger, Basel, p 21
Oswald WD (1982) Alltagsaktivitäten und die Speed/Power-Komponenten von Testleistungen. Z Geront 15:11–14
Oswald WD (1986) Der Zahlen-Verbindungs-Test im höheren Lebensalter. In: Daumenlang K, Sauer J (Hrsg) Aspekte psychologischer Forschung. Hogrefe, Göttingen, pp 377–388
Oswald WD (1990) Zur Diagnostik von alters- und krankheitsbedingten Gehirnleistungsstörungen. Zbl Neurol Psychiat 255:167
Oswald WD, Adler C, Rupprecht R, Bayer-Feldmann C, Barth P (1990) Subjektive Alternssymptome als Indikator für hirnorganische Psychosyndrome – Die Entwicklung der Nürnberger-Selbsteinschätzungs-Liste (NSL). Z Gerontopsychol Gerontopsychiatr 3:249–264
Oswald WD, Fleischmann UM (1992) Nürnberger-Alters-Inventar NAI. Testkasten und Manual, 3. Aufl. Universität Erlangen-Nürnberg, Nürnberg. Hogrefe (Testzentrale Stuttgart), Göttingen
Oswald WD, Gunzelmann Th (1991) Psychometrie und klinische Beurteilung. In: Oswald WD, Herrmann W, Kanowski S, Lehr UM, Thomae H (Hrsg) Gerontologie – Medizinische, psychologische und sozialwissenschaftliche Grundbegriffe, 2. Aufl. Kohlhammer, Stuttgart, S431–S455
Oswald WD, Roth E (1987) Der Zahlen-Verbindungs-Test ZVT, 2. Aufl. Hogrefe, Göttingen
Perez FI, Stump DA, Gray JRA, Hart VR (1976) Intellectual performance in multi-infarct dementia and Alzheimer's disease. A replication study. Can J Neurol Sci 3:181–187
Reisberg B, Ferris SH, de Leon MJ, Crook T (1982) The Global Deterioration Scale (GDS): an instrument for the assessment of primary degenerative dementia (PDD). Am J Psychiatry 139:1136–1139
Reitan RM (1958) Validity of the trail making test as an indicator of organic brain damage. Percept Mot Skills 8:271–276
Salthouse TA (1985) Speed of behavior and its implications for cognition. In: Birren JE, Schaie KW (eds) Handbook of the psychology of aging. van Nostrand Reinhold, New York, pp 400–426
Wagner O, Oesterreich K, Hoyer S (1987) Interdisziplinäre Forschungsstrategien

in der Gerontopsychiatrie. Untersuchungen und Ergebnisse bei dementiellen und depressiven Patienten. Z Gerontol 20:195–203

Correspondence: Dr. D. Oswald, University of Erlangen-Nürnberg, Institut für Psychologie II – Lehrstuhl IV, Nägelsbachstrasse 25, D-90152 Erlangen, Federal Republic of Germany

NGF and Alzheimer's disease: a model for trophic factor therapy in neurodegeneration

B. Knüsel and **F. Hefti**

Division of Neurogerontology, Andrus Gerontology Center, University of Southern California, Los Angeles, CA, U.S.A.

Summary

A review on recent research progress in neurotrophic factors and their implication for experimental therapy of Alzheimer's disease is given. The status of NGF mechanisms in AD is not fully understood, although NGF administration in animal studies is suggested to counter at cholinergic atrophy present in AD. Limited clinical trials have been performed with NGF in AD, and effective therapy in AD will require protection of vulnerable neuronal populations. The possibilities of NGF treatment of CNS diseases including AD are intracerebral infusion, slow-releasing implants, implantation of cells producing recombinant NGF or local injection of genetic material producing active fragments or small molecules that pass the blood-brain barrier. NGF administration is expected to improve functional performance of surviving cells, but larger clinical trials are needed to confirm preliminary encouraging results of neurotrophic factor therapy.

Introduction

Alzheimer's disease (AD) is one of the major neurological disorders affecting the older generation. Contrasting with the enormous toll that this disease puts on the patient, his or her caregivers, and society as a whole, is the present lack of an effective therapy. The defining feature of AD is the formation of structures in the brain which to the pathologist in postmortem examination are known as neuritic plaques and neurofibrillary tangles. Probably of more significant neurological relevance is the region-specific degeneration of neuronal populations, particularly in areas connected to the hippocampus (Price, 1986; Kosik, 1992, reviews). Most notable among these populations are the cholinergic neurons of the basal forebrain, cell which are critically involved in functions of memory and cognition (Olton, 1990; Olton et

al., 1991). Therapeutic approaches for AD have been aimed at increasing brain cholinergic function, however, with only minimal success. Probably such treatment does not overcome the significant loss of cholinergic neurons, particularly in more advanced stages of the disease. The degeneration of cholinergic (and other) neurons is not reduced by cholinergic agonists or inhibitors of acetylcholine breakdown. A different approach is to directly target neuronal degeneration by taking advantage of rather recently discovered trophic proteins which are present in the brain in minute amounts. These neurotrophic proteins are required for proper development of brain cells and are believed to play roles in the maintenance of function and structural integrity of neurons in the adult brain. Consequently, use of neurotrophic factor mechanisms has been proposed as a novel treatment approach to therapeutically counteract neurodegeneration in AD (Hefti and Schneider, 1989). This article summarizes and discusses some findings from our laboratory and from other which indicate that neurotrophic molecules, most notably nerve growth factor or NGF, might become useful in the fight against AD and other neurodegenerative diseases.

Neurotrophic factors

The research area of neurotrophic factors and its concepts emerged from developmental neurobiological studies by V. Hamburger, E. Bueker, R. Levi-Montalcini and S. Cohen, which led to the discovery that a specific protein, nerve growth factor (NGF), regulates survival and growth of neurons of the peripheral nervous system (see Levi-Montalcini, 1987). More recently developed ideas that neurotrophic factors are involved in neurodegeneration and the finding that many of the known growth factors are expressed in the brain brought fulminant growth to this field. This led to the discovery of actions on nerve cells by many novel proteins and by proteins previously known for actions on non-neuronal cells. There is no generally accepted definition for the term "neurotrophic factor". Historically, the term described proteins that are involved in the regulation of survival of neurons during development. The presently available body of data suggests a more inclusive definition to also cover molecules for which an action as target-derived factor has not been demonstrated but which have been found in experimental systems to have trophic effects on neurons. We recently suggested the following definition: "Neurotrophic factors are endogenous soluble proteins regulating survival, growth, morphological plasticity, or expression of differentiated functions of neurons" (Hefti et al., 1990). Particular interest in the past few years has been on a group of factors which have similarity to NGF and which include brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neuro-

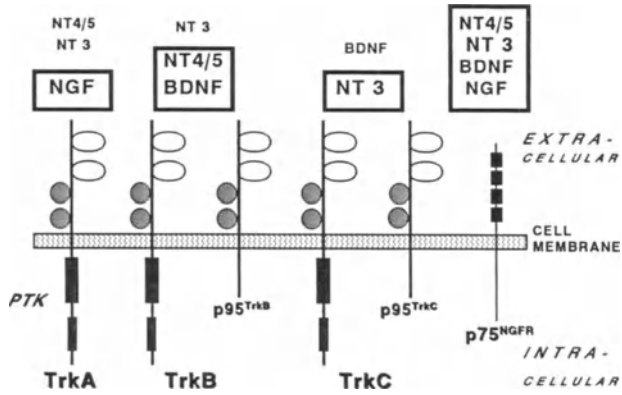

Fig. 1. The neurotrophins and their receptors. NGF, BDNF, NT-3 and NT-4/5 form a family of related proteins with approximately 50% amino acid sequence homology. Similarly, their receptors, the Trk type proteins or Trks, share the same degree of sequence homology. The different Trks all have one or two principal ligands (TrkA, NGF, TrkB, BDNF and NT-4/5; TrkC, NT-3) but other neurotrophins at higher concentrations can also bind to the same receptor. Some Trks belong to the category of transmembrane receptors of the protein tyrosine kinase type. Common to all these transducing receptors is the configuration into an extracellular ligand binding domain, a transmembrane domain and an intracellular domain with enzymatic protein kinase activity. Upon ligand binding, the receptors are phosphorylated on tyrosine which leads to activation of the enzymatic activity. The activated receptor is able to interact with various intracellular enzymes which together form a "signal transduction cascade", ultimately leading to the cellular neurotrophic stimulation. The activation of Trk receptors can be visualized by appropriate biochemical methods (see Figs. 3, 5). In addition to the transducing neurotrophin receptors, truncated forms of TrkB ($p95^{TrkB}$) and TrkC ($p95^{TrkC}$) have been found. Another protein which is known since several years is called the low affinity (p75) NGF receptor (P75NGFR). The functions of the truncated Trks and of p75NGFR which equally binds all neurotrophins are not known. *PTK* protein tyrosine kinase

trophin-4/5 (NT-4/5). NT-4/5 was discovered simultaneously in two laboratories and was first believed to be two differing proteins. Some of the neurotrophic factors, among them the neurotrophins have been shown to act through specific receptors on the surface of brain cells (Fig. 1). The complex events of the "signal transduction cascade" which is triggered intracellularly after binding of a neurotrophic factor to its receptor are only now being unravelled (Kaplan et al., 1993).

Development of NGF for experimental therapy of Alzheimer's disease

The decline of cholinergic function and cholinergic markers in the human AD septohippocampal and basalo-cortical projections (Davies and Maloney, 1976; Whitehouse et al., 1982; Etienne et al., 1986) is one

of the earliest changes in the disease (Francis et al., 1985) and the extent of the cholinergic deficit correlated well with the degree of memory impairment (DeKosky et al., 1992; Lehericy et al., 1993). The cholinergic changes observed in human AD are mimicked in the adult brain by lesions of the septo-hippocampal pathway which results in a gradual progression of events ranging from mild atrophy of the cholinergic cells to shrinkage associated with down-regulation of cell specific markers and finally to degeneration of the cell body (Daitz and Powell, 1954; Gage et al., 1986; Hefti, 1986; Williams et al., 1986; O'Brien et al., 1990; Hagg et al., 1988, 1989; Sofroniew et al., 1988). Different types of cholinergic lesion models are used by various investigators in Alzheimer's related research. Partial fimbrial transections are most often used in our laboratory, whereas complete transections are sometimes employed for comparison. The partial fimbrial transection, chosen as model several years ago (Hefti et al., 1984), leaves a tissue bridge between septum and hippocampus. Spared cholinergic axons projecting to the hippocampus allow the investigator to study actions of trophic factors on survival cholinergic terminals.

The discovery of the cholinergic cell loss in AD (Whitehouse et al., 1982) fortuitously coincided with the discovery that NGF is a neurotrophic factor for basal forebrain cholinergic cells. Cell culture studies provided initial evidence that NGF acts on these neurons (Honegger and Lenoir, 1982; Hefti et al., 1985) and they helped to characterize in detail the nature of the trophic effects (e.g., Hartikka and Hefti, 1988). Today the role of NGF in the developmental growth of basal forebrain cholinergic neurons and their continued responsiveness to this factor during adult life are well established (Thoenen et al., 1987; Hefti et al., 1989, reviews). We recently could establish at the level of the NGF receptor that adult rat brain tissue very strongly responds to

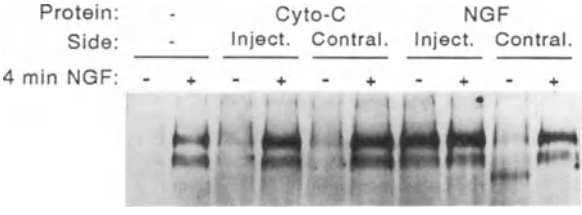

Fig. 2. NGF activates NGF receptors in adult striatum. Experimental rats were treated daily for one week with injections of 1 µg human recombinant NGF or cytochrome-C as control protein. 24 hours after the last injection microslices were prepared from the striatum and some of the slices were acutely treated with NGF (100 ng/ml) under physiological conditions. The extent of tyrosine phosphorylation of Trk type receptors was then analyzed by immunoprecipitation and western blotting (Knüsel et al., 1993). Trk type receptors from the injected side of animals receiving NGF were fully activated, even in absence of an acute treatment in the slice preparation, illustrating the effectiveness of the NGF injections in-vivo. Protein: Cytochrome-C or NGF used in daily injections

NGF and that this response is not diminished throughout adult life (Fig. 2 and Knüsel et al., 1993). A substantial body of data further indicates that NGF is effective in preventing cholinergic degeneration following experimental injury as described above or associated with normal aging. Based on these findings which were reviewed recently (Hefti et al., 1989; Hefti, 1993) NGF has been proposed for initial clinical trials in AD.

The status of NGF mechanisms in Alzheimer brain tissue remains to be clarified. While NGF levels remain constant or are elevated (Goedert et al., 1989; Phillips et al., 1991), many other steps in NGF synthesis and signal transduction may be affected in AD. If NGF receptor and transduction mechanisms in AD are sufficiently intact, the animal studies imply that NGF administration will counteract cholinergic atrophy, irrespective of the actual cause. Thus, administration of NGF to Alzheimer patients represents a pharmacologic attempt to induce hypertrophy of cholinergic neurons surviving in the Alzheimer brain, not a replacement therapy. NGF-induced hypertrophy in animal models manifests itself in increases in the expression of structural and transmitter-specific proteins, in a reversal of age-related degenerative changes and increased resistance to experimental insults, and in increased ability to influence postsynaptic cells. In consequence, NGF administration to Alzheimer patients is anticipated to attenuate the rate of degeneration of cholinergic neurons and improve the functional performance of the surviving cells. NGF treatment may then attenuate the deterioration or eventually improve the symptomatic behavioral changes that are a consequence of the cholinergic deficit (Hefti and Schneider, 1989, review). Limited clinical trials have been initiated and encouraging effects in a single Alzheimer patient have been reported (Olson et al., 1992). However, possible detrimental effects of intraventricular NGF treatment need to be considered. They include induction of aberrant cholinergic sprouting as observed in lesioned rodents (Williams et al., 1986). Elevation in brain levels of amyloid precursor protein mRNA in neonatal animals (Mobley et al., 1988) gave reason for concern, however, this induction seems to be limited to a mRNA species more abundant in juvenile brains and reduced in Alzheimer tissue (Ohyagi and Tabira, 1993). There could be undesired proliferation of NGF-responsive non-neuronal cells (Nakagawara et al., 1993), NGF infusions may act on intracerebral sympathetic neurons and produce changes in local blood flow (Isaacson et al., 1990), and hypophagia may occur as a side-effect of intraventricular NGF treatment (Williams, 1991).

Other neurotrophic factors and Alzheimer's disease

Despite the current focus on NGF, recent results suggest that other neurotropins might need to be considered with regard to the

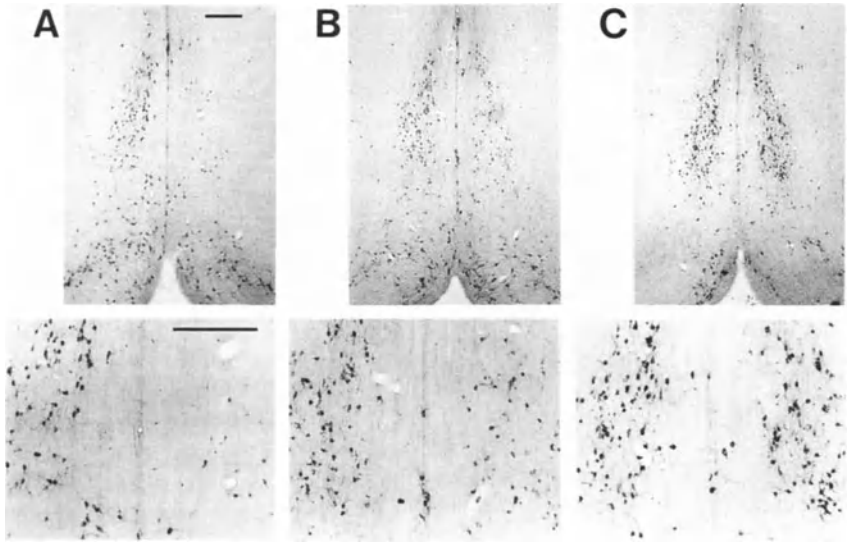

Fig. 3. NGF fully, BDNF partially protects cholinergic neurons of the basal forebrain from axotomy-induced degeneration. Rats received unilateral partial lesions of the fimbria, transecting cholinergic septo-hippocampal axons projecting through this pathway. After surgery, the rats received daily injections of 0.7 μg cytochrome-C control protein, of NGF or of BDNF. After three weeks the animals were sacrificed and the brain immunohistochemically processed to visualize cholinergic neurons. In cytochrome-C treated animals 41% of cholinergic cells remained on the lesioned side, while these numbers were 95% and 59% for the NGF and the BDNF treatments, respectively. The figure shows sections through the cholinergic area in the medial septum. The lower panels are higher magnifications of the upper panels. **A** cytochrome-C treated control; **B** BDNF treated; Cerebrolysin, NGF treated

cholinergic basal forebrain neurons and their involvement in AD. Based on cell culture findings (Alderson et al., 1990; Knüsel et al., 1991), rats with partial fimbrial transection were treated chronically with BDNF. Cholinergic cell loss after three weeks was significantly decreased in BDNF treated animals when compared to controls. The protective effect with BDNF treatment was not as high as with NGF and, unlike with NGF; ChAT seemed not upregulated in surviving cholinergic cells (Fig. 3; Knüsel et al., 1992a). While these findings establish a BDNF response in basal forebrain cholinergic neurons in the adult, the expression of BDNF itself in the normal hippocampus seems to be under cholinergic control. Partial and full fimbria-fornix transections results in a partial or almost complete loss of mRNA for BDNF in the hippocampus and a similar effect was observed after treatment with muscarinic but not nicotinic antagonists (Lapchak et al., 1993b). These results invite the speculation of a positive neurotrophic feedback loop in the intact brain involving basal forebrain cholinergic neurons and cells in the hippo-

campus. Most interestingly, in human AD hippocampus the mRNA for BDNF, but not for NGF and NT-3 is significantly decreased (Phillips et al., 1991). Such a neurotrophic feedback loop could be compromised in AD, further exacerbating the cholinergic deficit. Differences in the effects of NGF and BDNF indicate that the functions of these factors on basal forebrain cholinergic neurons are different (Knüsel et al., 1991, 1992a) suggesting that an optimal therapy in AD might require stimulation of both mechanisms by using combinations of neurotrophic factors or genetically engineered mutants which combine properties of more than one neurotrophin (Ibanez et al., 1991).

Further, maximally effective growth factor therapy of AD will require protection of all vulnerable neuronal populations, not just the cholinergic ones. There is initial evidence that ascending noradrenergic neurons of the entorhinal cortex are protected from degeneration induced by axotomy in rats (Cummings et al., 1992), a lesion modelling entorhinal cortical degeneration in Alzheimer's disease. CNTF protects adult thalamo-cortical neurons from degeneration after axotomy (Clatterbuck et al., 1993). Again, pluripotent growth factors or combinations of factors protecting various populations of vulnerable neurons may be necessary to obtain optimal behavioral effects. The identification of growth factors effectively preventing degeneration of cortical and hippocampal neurons represents one of the major challenges in the neurotrophic factor field.

Pharmacology of neurotrophic factors

Neurotrophic factors should become useful in the treatment of diseases involving structural disintegration of nervous tissue, since they can attenuate age related and experimentally induced degeneration and behavioral deficits in animals. There are several pharmacological strategies which could be pursued to pharmacologically exploit neurotrophic factor mechanisms. To be able to reach neuronal populations in the brain, neurotrophic factors will have to be given by *intracerebral infusion*, since these proteins do not cross the blood-brain barrier. In experimental animals and humans, NGF has been chronically infused into the brain with the help of a mechanical pump device (Koliatsos et al., 1991b; Olson et al., 1992). Sophisticated subcutaneous pumps are available which can be programmed and refilled by transcutaneous injections and which are adequately tolerated (Harbaugh et al., 1989). An additional factor to be considered is the biodistribution of neurotrophic factors following intraventricular infusion. Initial studies with NGF in rats revealed rapid distribution within the ventricular system but only very limited penetration into the brain parenchyma (Lapchak et al., 1993a) compatible with the predictions made from diffusion kinetics of proteins (Pardridge, 1991).

Given the difficulties with central administration of neurotrophic factors, there is an active search for alternative delivery strategies. *Slow-releasing implants* containing the active protein embedded in a biodegradable polymer matrix could be implanted in a single surgical session. NGF-containing polymers implanted into rat brains prevent the degeneration of cholinergic neurons induced by experimental lesions (Hoffman et al., 1990; Maysinger et al., 1992). It may be possible to deliver protein growth factors to the brain by modifying them chemically or packaging them so that they *pass the blood-brain barrier*. A conjugate of NGF and monoclonal anti-transferrin receptor antibodies is transported to the brain by binding to transferrin receptors (Friden et al., 1993). Further methods to be considered are cell therapy and gene therapy. Intracerebral *implantation of cells producing recombinant NGF* evokes trophic actions on NGF responsive cholinergic neurons similar to those observed after intraventricular infusion (Rosenberg et al., 1988). It will be possible to produce cells which do not proliferate after implantation, are well tolerated by the host, and in which the synthesis of the desired growth factor is under the control of a promoter regulated by drugs. The dosage of this systemically administered drug would control the synthesis of the neurotrophic factor in the brain. Growth factor producing cells can be encapsulated to avoid infiltration of brain tissue (Aebischer et al., 1991). Finally, it seems feasible to introduce the gene coding for trophic factors into targeted brain cells by *local injection of genetic material*. The production of *active fragments or small molecules* which pass into the brain and mimick the active sites of neurotrophic factors represents another possible approach. Fragments of bFGF, insulin-like growth factor I (IGF-I), and IGF-II appear to retain activity (Baird et al., 1988; Konishi et al., 1989). A synthetic peptide analogue of transforming growth factor-alpha (TGF-a) was shown to specifically inhibit EGF and TGF-a growth stimulatory actions on responsive cell lines (Eppstein et al., 1989). Effective concentrations of the active peptides were much higher than those of the native proteins, suggesting that they bind to some but not all of the sites forming the contact to the natural ligand. However, studies with peptide fragments and modified peptides will provide a basis for molecular modelling studies attempting to replace peptides with non-peptide effectors. The theoretical feasibility of this approach is illustrated by the example of endorphins and morphine which act on the same receptors.

Problems associated with the use of protein growth factors could be bypassed if it was possible to *manipulate critical steps in the synthesis, release and transduction mechanism of endogenous growth factors*. Characterization of regulatory sequences of neurotrophic factor genes may reveal possibilities for direct pharmacological manipulation of gene expression (e.g., D'Mello and Heinrich, 1991; Shintani et al., 1993). There is emerging evidence that non-protein hormones are

involved in the regulation of neurotrophic factor expression, for example, glucocorticoids prevent the induction of NGF in peripheral nerves (Lindholm et al., 1990a), brain levels of NGF are affected by testosterone (Katoh-Semba et al., 1990), and estrogen receptors on basal forebrain cholinergic neurons mediate the downregulation of the p75 NGF receptor protein (Toran-Allerand et al., 1992; Gibbs and Pfaff, 1992). In absence of an understanding of their mechanism of action, several compounds were found to regulate NGF synthesis, including catechol derivates and complex phenols called hericones (Takeuchi et al., 1990; Kawagishi et al., 1991; Carswell et al., 1992). NGF-mimicking actions were described for long-chain fatty acids, 1, 1, 3-tricyano-2-amino-1-propene (triap), and sesquiterpene-neoligans (Borg et al., 1987; Paul and DaVanzo et al., 1992; Fukuyama et al., 1992). Besides analogues of neurotrophic factors, which mimic the confirmation of the natural protein ligand and interact with the same binding site, it may be possible to find drugs which influence neurotrophic factor receptor function by binding to parts different from those interacting with the proteins. This situation would be an analogy to the benzodiazepine modification of GABA receptor function, or recently made the incidental observation that K-252b, a compound which was known to inhibit the actions of NGF and other neurotrophins, at much lower concentration than required for this inhibition, significantly enhanced the response of basal forebrain cholinergic neurons to NT-3 (Knüsel et al., 1992b; Knüsel and Hefti, 1992). In absence of K-252b, NT-3 produced a very small increase in ChAT activity as compared to the stimulatory effects of NGF or BDNF in this system. Low concentrations of K-252b potentiated the NT-3 response to the same level as achieved by NGF or BDNF (Fig. 3). Dose-response analysis for NT-3 revealed that K-252b increased both, potency and maximal efficacy of the trophic action of NT-3 on the basal forebrain cholinergic neuron but could also be observed in other neurons in culture and in the pheochromocytoma PC12 cell line which is used in many studies on neuronal growth and differentiation (Knüsel et al., 1992b). More recently we could confirm this effect also in adult brain tissue of rat hippocampus and striatum (Fig. 4). Microslices of striatum and hippocampus were prepared and incubated under physiological conditions with low and high concentrations of K-252b and with NGF or NT-3 as indicated in Fig. 5. While the high concentration of the drug, as expected, completely inhibited the effect of NGF on Trk tyrosine phosphorylation, the low concentration very markedly increased the effect of NT-3. It remains to be seen whether a similar effect of K-252b can be observed in the intact animal. The ultimate goal of this study will be to enhance the action of endogenous NT-3 in the brain where it is found predominantly in the hippocampus (Phillips et al., 1990), one of the target areas of the basal forebrain cholinergic neurons.

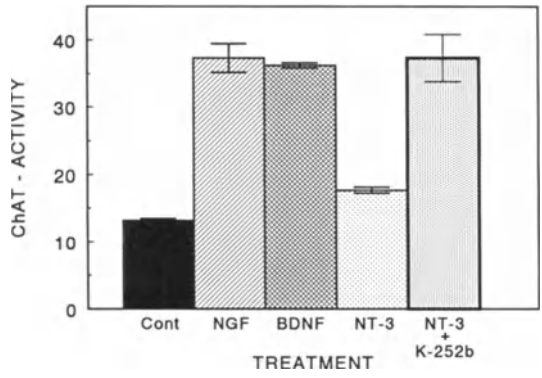

Fig. 4. K-252b enhances the action of NT-3 on cholinergic neurons in cultures of basal forebrain neurons. Cultures were prepared from dissociated neurons of E15 embryonic basal forebrain. The cultures were grown for 7 days and treated for the last 5 days with factors and K-252b as indicated. The cultures were then taken for biochemical determination of the activity of the cholinergic marker enzyme choline acetyltransferase (ChAT). Growth factor concentrations producing maximal elevations of ChAT activity were used. Error bars represent SEMs. NT-3 by itself had only minimal stimulatory effect on the cholinergic brain neurons in culture. Copresence of 10 nM K-252b increased the effect of NT-3 to the levels of NGF and BDNF

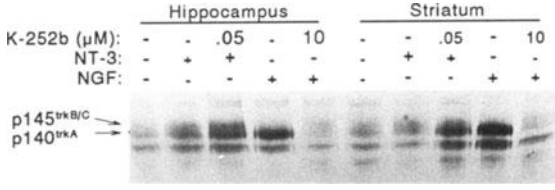

Fig. 5. Enhancement of the NT-3 action on brain cells by K-252b can be observed in adult brain tissue. Microslices were prepared from hippocampus and striatum of adult rats. Incubation of the slices was for 4 min. with NT-3 (200 ng/ml), with NT-3 and 30 nM K-252b, with NGF (100 ng/ml) or with NGF and 10 μM K-252b. The extent of tyrosine phosphorylation of Trk type receptors was then analyzed by immunoprecipitation and western blotting (Knüsel et al., 1993). While the high concentration of K-252b completely inhibited the response the NGF, the low concentration clearly enhanced the response to NT-3 in tissue from hippocampus as well as striatum. $p145^{TrkB/C}$, transducing TrkB and TrkC neurotrophin receptors; $p140^{TrkA}$, transducing NGF receptor

Outlook

In contrast to some neurodegenerative diseases which involve selective loss of a single population of neurons (e.g. Parkinson's disease and the dopaminergic neurons of the substantia nigra). In AD there is region-specific loss of multiple neuronal populations, particularly in areas connected to the hippocampus. Strongly affected are hippocampal inputs from the entorhinal cortex, the cholinergic basal fore-

brain and the noradrenergic locus coeruleus. However, there has been increasing awareness that the selective loss of neuronal populations determines the behavioral symptoms of AD (DeKosky et al., 1992; Lehericy et al., 1993). Coincidentally, this is the population of brain cells for which the existence of a highly effective neurotrophic factor has been demonstrated in animal studies, including studies in primates (Tuszynski et al., 1991; Koliatsos et al., 1991). It is expected that NGF administration to Alzheimer patients will attenuate the rate of degeneration of cholinergic neurons and improve the functional performance of the surviving cells. If larger clinical trials with NGF confirm the preliminary encouraging results (Olson et al., 1992), this will represent an important validation of the new concept of neurotrophic factor therapy for neurodegeneration. NGF in AD is not the only example of current clinical interest in neurotrophic factor therapy for neurodegeneration. NGF in AD is not the only example of current clinical interest in neurotrophic factors. At present, there are also ongoing clinical trials with neurotrophic factors for peripheral sensory neuropathy and amyotrophic lateral sclerosis (Hefti, 1993). These trials document that the concepts of neurotrophic mechanisms have finally found their way into preliminary clinical practicality. However, there is no approved product yet. "Neurotrophic factor therapy" has yet to deliver on its promises. Many investigators feel that the rush to clinical trials has left research on basic mechanisms trailing behind (Touchett, 1993). However, it should not be forgotten that basic research cannot offer absolutely certain predictions and that ultimately only clinical experimentation is able to ascertain efficacy. There are examples of drugs which found their indications based on clinical observations, in particular, neuroactive drugs like benzodiazepines, antidepressants and neuroleptics. Clinical use of these drugs stimulated basic research in underlying mechanisms and played crucial parts in the analysis of neurotransmitter receptors and transport systems, results which then led to improved versions of drugs. It is hoped that the clinical use of neurotrophic factors will stimulate research in a similar fashion. The neurotrophic factor field hold unique promise for better understanding of mechanisms of nervous system degeneration and function in adult of aging individuals. While still at an embryonic stage, neurotrophic factor therapy appears to be one of the most promising approaches towards effective treatment of neurodegenerative diseases. Accordingly, the results of the first clinical trials are eagerly awaited both by medical researchers and by clinicians.

References

Aebischer P, Winn SR, Tresco PA, Jaeger CB, Greene LA (1991) Transplantation of polymer encapsulated neurotransmitter secreting cells: effect of the encapsulation technique. J Biomech Eng 113:178–183

Alderson RF, Alterman AL, Barde YA, Lindsay RM (1990) Brain derived neurotrophic factor increases survival and differentiated functions of rat septal cholinergic neurons in culture. Neuron 5:297–306

Baird A, Schubert D, Ling N, Guillemin R (1988) Receptor- and heparin-binding domains of basic fibroblast growth factor. Proc Natl Acad Sci USA 85:2324–2328

Borg J, et al (1987) Neurotrophic effect of naturally occurring long-chain fatty alcohols on cultured CNS neurons. FEB 213:406–410

Carswell S, Hoffmann EK, Clopton-Hartpence K, Wilcox HM, Lewis ME (1992) Induction of NGF by isoproterenol, 4-methylcatechol and serum occurs by three distinct mechanisms. Mol Brain Res 15:145–150

Clatterbuck RE, Price DL, Koliatsos VE (1993) Ciliary neurotrophic factor prevents retrograde neuronal death the adult central nervous system. Proc Natl Acad Sci 90:2222–2226

Cummings BJ, Yee GJ, Cotman CW (1992) bFGF promotes the survival of entorhinal layer II neurons after perforant path axotomy. Brain Res 591:271–276

D'Mello SR, Heinrich G (1991) Structural and functional identification of regulatory regions and cis elements surrounding the nerve growth factor gene promotor. Mol Brain Res 11:255–264

Daitz HM, Powell TPS (1954) Studies on the connections of the fornix system. J Neurol Neurosurg Psychiatry 17:75–82

Davies P, Maloney AJF (1976) Lancet II:1403

DeKosky ST, Harbaugh RE, Schmitt FA, Bakay RAE, Chui HC, Knopman DS, Reeder TM, Shetter AG, Senter HJ, Markesbery WR and the Intraventricular Bethanechol Study Group (1992) Cortical biopsy in Alzheimer's disease: diagnostic accuracy and neurochemical, neuropathological, and cognitive correlations. Ann Neurol 32:625–632

Eppstein DA, Marsh YV, Schryver BB, Bertics PJ (1989) Inhibition of epidermal growth factor/transforming growth factor-alpha-stimulated cell growth by a synthetic peptide. J Cell Physiol 141:420–430

Etienne P, Robitaille Y, Wood P, Gauthier S, Nair NP, Quirion R (1986) Nucleus basalis neuronal loss, neuritic plaques and choline acetyltransferase activity in advanced Alzheimer's disease. Neuroscience 19:1279–91

Francis PT, Palmer AM, Sioms NR, Bowen DM, Davison AN, Esiri MM, Neary D (1985) Neurochemical studies of early-onset Alzheimer's disease. Possible influence on treatment. Lancet 4:7–11

Friden PM, Walus LR, Watson P, Doctrow SR, Kozarich JW, Backman C, Bergman H, Hoffer B, Blomm F, Granholm AC (1993) Blood-brain barrier penetration and in vivo activity of an NGF conjugate. Science 259:373–376

Friedman WJ, Ibanez CF, Hallbook F, Persson H, Cain LD, Dreyfus CF, Black LB (1993) Differential actions of neurotrophins in the locus coeruleus and basal forebrain. Exp Neurol 119:72–78

Fukuyama Y, Otoshi Y, Nakamura K, Kodama M, Nagasawa M, Hasegawa T, Okazaki H, Sugawara M (1992) Neurotrophic sesquiterpene-neoligans from Magnolia obovata: structure and neurotrophic activity. Tetrahedron 48:377–392

Gage FH, Wictorin K, Fischer W, Williams LR, Varon S, Bjorklund A (1986) Life and death of cholinergic neurons: in the septal and diagnoal band region following complete fimbria formix transections. Neuroscience 19:241–256

Gether U, Johanson TE, Snider RM, Lowe JA, Nakanishi S, Schwartz TW (1993) Different binding epitopes on the NK1 receptor for substance P and non-peptide antagonist. Nature 362:345–348

Gibbs RB, Pfaff DW (1992) Effects of estrogen and fimbria/fornix transection on p75NGFR and ChAT expression in the medial septum and diagonal band of Broca. Exp Neurol 116:23–39

Goedert M, Fine A, Dawbarn D, Wilcock GK, Chao MV (1989) Nerve growth factor receptor mRNA distribution in human brain: normal levels in basal forebrain in Alzheimer's disease. Mol Brain Res 5:1–7

Hagg T, Fass-Holmes B, Vahlsing HL, Manthorpe M, Conner JM, Varon S (1989) Nerve growth factor (NGF) reverses axotomy-induced decreases in choline acetyltransferase, NGF receptor and size of medial septum cholinergic neurons. Brain Res 505:29–38

Hagg T, Manthorpe M, Vahlsing HL, Varon S (1988) Delayed treatment with nerve growth factor reverses the apparent loss of cholinergic neurons after acute brain damage. Exp Neurol 101:303–312

Harbaugh RE, Reeder TM, Senter HJ, Knopman DS, Baskin DS, et al (1989) Intracerebroventricular bethanechol chloride infusion in Alzheimer's disease: results of a collaborative double-blind study. J Neurosurg 71:481–486

Hartikka J, Hefti F (1988) Comparison of nerve growth factor's effects on development of septum, striatum, and nucleus basalis cholinergic neurons in vitro. J Neurosci Res 21:352–364

Hefti F (1986) Nerve growth factor (NGF) promotes survival of septal cholinergic neurons after fimbrial transections. J Neurosci 6:2155–2162

Hefti F (1993) Neurotrophic factor therapy for nervous system degenerative diseases. Neuron (in press)

Hefti F, Schneider LS (1989) Rationale for the planned clinical trials with nerve growth factor in Alzheimer's disease. Psych Dev 4:297–315

Hefti F, David A, Hartikka J (1984) Chronic intraventricular injections of nerve growth factor elevate hippocampal choline acetyltransferase activity in adult rats with partial septo-hippocampal lesions. Brain Res 293:305–311

Hefti F, Hartikka J, Eckenstein F, Gnahn H, Heumann R, Schwab M (1985) Nerve growth factor (NGF) increases cholinergic acetyltransferase but not survival or fiber outgrowth of cultured fetal septal cholinergic neurons. Neuroscience 14:55–68

Hefti F, Hartikka J, Knüsel B (1989) Function of neurotrophic factors in the adult and aging brain and their possible use in the treatment of neurodegenerative diseases. Neurobiol Aging 10:515–533

Hoffman D, Wahlberg L, Aebischer P (1990) NGF released from a polymer matrix prevents loss of ChAT expression in basal forebrain neurons following a Fimbria-Fornix lesion. Exp Neurol 110:39–44

Honegger P, Lenoir D (1982) Nerve growth factor (NGF) stimulation of cholinergic telencephalic neurons in aggregating cell cultures. Dev Brain Res 3:229–239

Ibanez CF, Hallbook F, Ebendal T, Persson H (1990) Structure-function studies of nerve growth factor: functional importance of highly conserved amino acid residues. EMBO J 9:1477–1483

Ip NV, McClain J, Barrezueta NX, Aldrich TH, Pan L, Li Y, Wiegand SJ, Friedman B, Davis S, Yancopoulos G (1993a) The alpha component of the CNTF receptor is required for signaling and defines potential CNTF targets in the adult and during development. Neuron 10:89–102

Isaacson LG, Saffran BN, Crutcher KA (1990) Intracerebral NGF infusion induces hyperinnervation of cerebral blood vessels. Neurobiol Aging 11:51–55

Kaplan DR, Perkins A, Morrison DK (1992) Signal transduction by receptor tyrosine kinases. In: Mc Guire WL (ed) Oncogenes and tumor suppressor genes in human malignancies. Kluwer Academic Publishers, Norwell MA

Katoh-Semba R, Semba R, Kashiwamata S, Kato K (1991) Elevated concentrations of beta-nerve growth factor in selected tissues from senescence-accelerated mice (SAM-P/8). Mech Ageing Dev 59:163–175

Kawagishi H, Ando M, Sakamoto H, Yoshida S, Ojima F, Ishiguro Y, Ukai N, Furokawa S (1991) Hericenones cerebrolysin, D and E, stimulators of nerve growth factor (NGF)-synthesis, from the mushroom Hericium erinaceum. Tetrahedron Lett 32:4561–4564

Knüsel B, Hefti F (1992) K-252 compounds: modulators of neurotrophin signal tranduction. J Neurochem 59:1987–1996

Knüsel B, Winslow JW, Rosenthal A, Burton LE, Seid DP, Nikolics K, Hefti F (1991) Promotion of central cholinergic and dopaminergic neuron differentiation by brain-derived neurotrophic factor but not neurotrophin-3. Proc Natl Acad Sci USA 88:961–965

Knüsel B, Beck Kd, Winslow JW, Rosenthal A, Burton LE, Widmer HR, Nikolics K, Hefti F (1992a) Brain derived neurotrophic factor administration protects basal forebrain cholinergica but not nigral dopaminergic neurons from degenerative changes after axotomy in the adult rat brain. J Neurosci 12: 4391–4402

Knüsel B, Kaplan DR, Winslow JW, Rosenthal A, Burton LE, Beck KD, Rabin S, Nikolics K, Hefti F (1992b) K-252b selectively potentiates cellular actions and trk tyrosine phosphorylation mediated by neurotrophin-3. J Neurochem 59:715–722

Knüsel B, Rabin S, Hefti F, Kaplan DR (1994) Regulated neurotrophin receptor responsiveness during neuronal migration and early differentiation. J Neurosci 14:1542–1554

Koliatsos VE, Clatterbuck RE, Nauta HJW, Knüsel B, Burton LE, Hefti F, Mobley WC, Price DL (1991) Human nerve growth factor prevents degeneration of basal forebrain cholinergic neurons in primates. Ann Neurol 30:831–840

Koliatsos VE, Clatterbuck RE, Winslow JW, Cayouette MH, Price DL (1993) Evidence that brain-derived neurotrophic factor is a trohic factor for motor neurons in vivo. Neuron 10:359–367

Konishi Y, Kotts CE, Bullock LD, Tou JS, Johnson DA (1989) Fragments of bovine insulin-like growth factors I and II stimulate proliferation of rat L6 myoblast cells. Biochemistry 28:8872–8877

Kosik KS (1992) Alzheimer's disease: a cell biological perspective. Science 256:780–783

Lapchak PA, Araujo DM, Carswell S, Hefti F (1993a) Distribution of (^{125}I) nerve growth factor in the rat brain following an intraventricular injection: sequestration by trkA mRNA expressing septal neurons. Neuroscience 52:575–585

Lapchak PA, Araujo DM, Hefti F (1993b) Cholinergic regulation of hippocampal brain-derived neurotrophic factor mRNA expression: evidence from lesion and chronic cholinergic drug treatment studies. Neuroscience 53:379–394

Lehericy S, Hirsch EC, Cervera-Pierot P, Hersh LB, Bakchine S, Piette F, Duyckaerts Cerebrolysin Hauw JJ, Javoy-Agid F, Agid Y (1993) Heterogene-

ity and selectivity of the degeneration of cholinergic neurons in the basal forebrain of patients with Alzheimer's disease J Comp Neurol 330:15–31

Levi-Montalcini R (1987) The nerve growth factor: thirty-five years later. EMBO J 6:1145–1154

Lindholm D, Hengerer B, Heumann R, Carroll P, Thoenen H (1990) Glucocorticoid hormones negatively regulate nerve growth factor expression in vivo and in cultured rat fibroblasts. Eur J Neurosci 2:795–801

Maysinger D, Jalsenjak I, Cuello AC (1992) Microencapsulated nerve growth factor: effects on the forebrain neurons following devascularizing cortical lesions. Neurosci Lett 140:71–74

Mobley WC, Neve RL, Prusiner SB, McKinley MP (1988) Nerve growth factor induces gene expression for prion- and Alzheimer's beta-amyloid proteins. Proc Natl Acad Sci USA 85:9811–9815

Nakagawara A, Arima-Nakagawara M, Scavarda NJ, Azar CG, Cantor AB, Brodeur GM (1993) Association between high levels of expression of the trk gene and favorable outcome in human neuroblastoma. N Engl J Med 328:847–854

O'Brien TS, Svendsen CN, Isacson O, Sofroniew MV (1990) Loss of true blue labelling from the medial septum following transection of the fimbria-fornix: evidence for the death of cholinergic and non-cholinergic neurons. Brain Res 508:249–256

Ohyagi Y, Tabira T (1993) Effect of growth factors and cytokines on expression of amyloid beta protein precursor mRNAs in cultured neural cells. Mol Brain Res 18:127–132

Olson L, Nordberg A, von Holst H, Backman L, Ebendahl T, Alafuzoff I, Amberla K, Hartvig P, Herlitz A, Lilja A, Lundquist H, Langstron B, Meyerson B, Persson A, Viitanen M, Winblad B, Seiger A (1992) Nerve growth factor affects 11C-nicotine binding, blood flow, EEG and verbal episodic memory in an Alzheimer patient. J Neural Transm [P-D Sect] 4:79–95

Olton DS (1990) Dementia animal models of the cognitive impairments following damage to the basal forebrain cholinergic system. Brain Res Bull 25:499–502

Olton D, Markowska A, Voytko ML, Givens B, Gorman L, Wenk G (1991) Basal forebrain cholinergic system: a functional analysis. Adv Exp Med Biol 295:353–72

Pardridge WM (1991) Transnasal and intraventricular delivery of drugs. In: Pardridge WM (ed) Peptide drug delivery to the brain. Raven Press, New York, pp 99–122

Park TH, Mytilineou C (1992) Protection from 1-methyl-4-phenylpyridinium (MPP+) toxicity and stimulation of regrowth of MPP +-damaged dopaminergic fibers by treatment of mesencephalic cultures with EGF and basic FGF. Brain Res 599:83–97

Paul JW, DaVanzo JP (1992) 1,1,3 Tricyano-2-amino-1 propene (Triap) stimulates choline acetyltransferase activity in vitro and in vivo. Dev Brain Res 67:113–120

Phillips HS, Hains JM, Laramee GR, Rosenthal A, Winslow JW (1990) Widespread expression of BDNF but not NT3 by target areas of basal forebrain cholinergic neurons. Science 250:290–292

Phillips HS, Hains JM, Armanini M, Laramee GR, Johnson SA, Winslow JW (1991) BDNF mRNA is decreased in the hippocampus of individuals with Alzheimer's disease. Neuron 7:695–702

Price DL (1986) New perspectives on Alzheimer's disease. Ann Rev Neurosci 9:489–512

Rosenberg MB, Friedmann T, Robertson RC, Tuszynksi M, Wolff JA, Breakfield XO, Gage FH (1988) Grafting genetically modified cells to the damaged brain: restorative effects of NGF expression. Science 242:1575–1578

Shintami A, Ohno Y, Kaisho Y, Sasada R, Igarashi K (1993) Identification of the functional regulatory region of the neurotrophin-3 gene promotor. Mol Brain Res 17:129–134

Sofroniew MV, Isacson O (1988) Distribution of degeneration of cholinergic neurons in the septum following axotomy in different portions of the fimbria-fornix: a correlation between cell loss and proximity to the lesion. J Chem Neuroant 1:327–337

Takeuchi R, Murase K, Furukawa Y, Furukawa S, Hayashi K (1990) Stimulation of nerve growth factor synthesis/secretion by 1, 4-benzoquinone and its derivatives in cultured mouse astroglial cells. FEBS Lett 261(1):63–66

Thoenen H, Bandtlow C, Heumann R (1987) The physiological function of nerve growth factor in the central nervous system: comparison with the periphery. Rev Physiol Biochem Pharmacol 109:145–178

Toran-Allerand CD, Miranda RC, Bentham WDL, Shrabji F, Brown TJ, Hochberg RB, MacLusky NJ (1992) Estrogen receptors colocalize with low-affinity nerve growth factor receptors in cholinergic neurons of the basal forebrain. Proc Natl Acad Sci USA 89:4668–4672

Touchette N (1993) Rising spirits for motor neuron revival. J NIH Res 5:53–59

Tuszynski MHUHS, Yoshida K, Gage FH (1991) Recombinant human nerve growth factor infusions prevent cholinergic neuronal degeneration in the adult primate brain. Ann Neurol 30:625–636

Whitehouse PJ, Price DL, Struble RG, Clark AW, Coyle JT, De Long MR (1982) Alzheimer's disease and senile dementia; loss of neurons in the basal forebrain. Science 215:1237–1239

Williams LR (1991) Hypophagia is induced by intracerebroventricular administration of nerve growth factor. Exp Neurol 113:31–37

Williams LR, Varon S, Peterson GM, Wictorin K, Fischer W, Bjorklund A, Gage FH (1986) Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. Proc Natl Acad Sci USA 83:9231–9235

Correspondence: Dr. B. Knüsel, Division of Neurogerontology, Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089-0191, U.S.A.

Efficacy of Cerebrolysin® in Alzheimer's disease

E. Ruether[1], **R. Ritter**[1], **M. Apecechea**[1], **S. Freitag**[1], and **M. Windisch**[2]

[1] Department of Psychiatry, University of Göttingen, School of Medicine, Göttingen, Federal Republic of Germany
[2] Department of Research & Development, Ebewe Pharmaceuticals Ltd, Unterach, Austria

Summary

Background. Alzheimer's disease (AD) accounts for about half of all cases of senile dementia. Its prevelance increases steeply with age, from less than 1% at 60 to 15% in the 9th decade. The disease causes great suffering to patients and their families, and huge costs to society. Four million patients suffer from AD in the USA alone.

Cerebrolysin is a nootropic drug directly affecting cerebral neurons due to a unique neurotrophic activity. Positive effects are expected in AD.

Methods. A prospective, double-blind, placebo-controlled, randomized trial was conducted. 120 subjects with mild to moderate dementia were included. Either placebo i.v. or 30 ml Cerebrolysin i.v. were given daily Monday to Friday for four weeks. The results were assessed with scales including the Clinical Global Impression (CGI), the SCAG (Sandoz Clinical Assessment-Geriatric), a trail-making test (ZVT-G), a self-assessment test (Bf/S), and the NAA which measures the activities of daily living.

Results. Cerebrolysin-treated subjects demonstrated marked improvement which was both clinically relevant and statistically significant. The responder rate in the Cerebrolysin group was 61.7%. There were no side effects or drop-outs in either group.

Conclusions. Cerebrolysin leads to fast and clinically relevant improvement in AD.

Introduction

Background. Alzheimer's disease (AD) is the most common and important degenerative disease of the brain. It was first described by

Alois Alzheimer in 1907. Although AD was originally characterized in elderly patients, it can occur in every period of adult life. However, the vast majority of Alzheimer victims are older than 60 (Skoog et al., 1993). The disease accounts for 60% of elderly patients in psychiatric hospitals. The annual incidence is 103 cases per 100 000 (Schoenberg et al., 1987).

The familial occurrence of AD has been well documented (Corder et al., 1993; Travis, 1993; Goate et al., 1991). However, the disease cannot be accounted for by a single gene (Breitner et al., 1991; Bergerm, 1993).

Forgetfulness is the major symptom (Alzheimer, 1911). The onset of mental changes is usually insidious. The illness develops gradually. Eventually the patient forgets how to use common objects and tools and even his or her name or the spouse's name. The disease may end in a poorly organized paranoid delusional state and leads to death. The course of this tragic illness extends usually over a period of five or more years. Surprisingly, although the intellectual capabilities of the victim deteriorate, corticospinal and corticosensory functions as well as visual acuity and visual fields remain intact (Davis et al., 1976; Perry et al., 1978).

The brain presents a diffuse atrophic appearance. Neurones are reduced in number, especially in cholinergic areas. Residual nerve cells are believed to loose dendrites. Three microscopic changes are characteristic:

1. deposits of amorphous material (senile plaques)
2. the presence of fibre-like strands within nerve cells (neurofibrillary tangles)
3. granulovacuolar degeneration of neurones, most evident in the pyramidal cell layer of the hippocampus.

The senile plaques contain amyloid, and the neurofibrillary tangles contain abnormally phosphorylated tau proteins.

Standard therapy. There is no standard therapy for AD. The loss of cholinergic neurons has led to clinical trials with anti-cholinergic drugs. Unfortunately the clinical efficacy could not be proven and severe adverse effects were seen (e.g. with tacrine) (Davidson et al., 1991; Kumar et al., 1991; Molloy et al., 1991).

Disturbances of other neurotransmitters, especially of the norepinephrine and serotonin systems (Mann et al., 1984; Davies, 1991), have led to therapeutic attemps with MAO-B blockers (Finali et al., 1991; Mangoni et al., 1991). The results were not encouraging. General nootropic drugs have led to slight improvement in clinical trials (Heiss et al., 1988), but their relevance is under dispute. Some attempts, e.g. with gangliosides (Svennerholm et al., 1990), were withdrawn because of severe side effects (Arzneimittelinformation Berlin, 1991, 1994). Disturbances of calcium metabolism in the brain led to the

assumption that calcium blockers might be clinically effective (Hoyer et al., 1992). Their efficacy is still to be proven (Jarvik, 1991). In summary it can be said that no drug has established itself in the therapy of AD.

A new approach is the clinical use of neurotrophic growth factors which is logical since the degeneration of neuronal systems is a characteristic feature of this disease (Gauthier et al., 1991). The association between the nerve growth factor (NGF) and cholinergic cell death was postulated by Hefti (1983). Various trials were designed to examine this possibility but they failed because neurotrophic factors are unable to pass the blood-brain-barrier (BBB) (Hefti et al., 1991; Tuszynski et al., 1990).

Cerebrolysin®. These two facts, the assumption that neurotrophic stimulation will be helpful in AD and that naturally occurring neurotrophic factors cannot cross the BBB, led to the therapeutic trial with Cerebrolysin®. This is a nootropic drug, containing biologically active peptides which act inside the brain and exert a neurotrophic effect. Cerebrolysin® is produced by a standardized controlled enzymatic breakdown of lipid-free porcine brain protein. It consists of free amino acids and peptides with a molecular weight of less than 10 kDa. Previous research showed that the action of Cerebrolysin® is brain-specific and due to the peptide fraction of the drug (Piswanger et al., 1990).

Cerebrolysin® exerts three effects on brain cells:

(1) neurotrophic effect (Albrecht et al., 1992)
(2) regulatory effect on neuronal metabolism (Windisch et al., 1987)
(3) influence on synaptic plasticity and transmission (Baskys et al., 1992)

The drug was designed in Austria, the neurotrophic effect was discovered by an independent research group in Japan (Shimazu et al., 1992, 1991). Many others have confirmed the unique neurotrophic activity of Cerebrolysin®. Animal models of human disease mimicking neurodegeneration showed that the peripheral application of Cerebrolysin® protects cholinergic neurons from cell death after fimbria-fornix transsection (Akai et al., 1992)

Astonishingly there are no modern clinical trials with this interesting drug. Some elder trials support the assumptions underlying this study (Hebenstreit et al., 1986; Suchanek-Fröhlich et al., 1986; Kofler et al., 1989, 1990a,b; Veresnchagin et al., 1991), however they do not satisfy up-to-date design specifications. The clinical efficacy of Cerebrolysin® was therefore examined in this trial.

Aim. The aim of the trial was to determine whether or not Cerebrolysin® is able to improve the clinical symptomatology and the cognitive performance of AD victims. Changes in CGI, SCAG, and trail-making test were used as primary variables; self-assessment and im-

provement in the activities of daily living were analyzed as secondary variables.

Patients and methods

Design. The trial was designed as a randomized, double-blind, placebo-controlled prospective study. Prior to the start, a case number evaluation was carried out, the underlying assumption being 40 and 70% responders in the placebo and Cerebrolysin® groups, respectively. A drop-out rate of 20% was estimated.

Weekly monitoring and quality auditing by an independent institution were integral parts of the design. The design was formulated according to the "Recommendations for the Evaluation of Nootropic Drugs" (Kanowski et al., 1988) and the "Recommendations of the Concensus Conference on the Methodology of Clinical Trials for Nootropics" (Amaducci et al., 1990). Design and informed consent forms were cleared by the Ethics Commitee in Freiburg. Informed consent was ob tained from the patient or a family member.

Patients. 120 patients were recruited between August and December 1991 who were randomized into two groups of 60 subjects each. There were no significant differences between the two groups at the beginning of the trial; 46% of all patients received therapy for accompanying illnesses. The study was carried out in four nursing homes and in the private practice of a GP in former West Germany.

Inclusion criteria. Male and female patients between 55 and 85 years of age with a diagnosis of senile dementia of the Alzheimer's type (SDAT) according to DSM III-R (American Psychiatric Association, 1987) were included. The subjects had mild to moderate disease according to the Global Deterioration Scale of Reisberg (GDS) (Reisberg et al., 1982). The Hachinski Ischaemic Score (Hachinski et al., 1975) had to be equal or less than four and the CT findings not contradictory to the diagnosis. The Mini Mental State Examination (MMSE) of Folstein had to be between 15 and 25 (Folstein et al., 1975).

Exclusion criteria. Drug and alcohol abuse, temporal organic psychosis and multimorbidity were the main reasons for exclusion.

Medication. The treated group received 30 ml of Cerebrolysin® (three ampoules) in 100 ml physiological saline i.v.. The placebo group received 30 ml of placebo (three ampoules) in 100 ml of physiological saline i.v. The infusions were given Monday to Friday for four weeks. The ampoules of Cerebrolysin® and placebo were identical. Yellow opaque infusion bottles were used to secure the blinding of the trial. The infusions were prepared by personell otherwise not involved in the study.

Assessment. All subjects were examined on the first day of the trial, after two weeks and at the end of the study. H&P including EKG and neurological examination was carried out on admission day, as well as blood and urinary chemistries. Blood pressure and heart rate were recorded daily before and after the infusion.

Grading by GDS and MMSE was carried out on admission day. A check list was used to record the symptoms. The mood was assessed with the Hamilton

Depression Scale (HAMD) (Hamilton, 1960). The SCAG (Shader et al., 1974), Clinical Global Impression (Guy et al., 1970) and the trail-making test (ZVT-G) (Oswald et al., 1986) were performed. The activities of daily living were assessed with the NAA (Nuremberg activities of daily living scale for the elderly). The self-assessement was recorded with the Bf/S scale (Zerssen et al., 1980). Adverse events were registered on the standardized international forms of DOTES and TWIS.

Statistics. Interval-scaled and symmetrically distributed samples with homogeneous variances were subjected to the t-test. In these tests ordinal scaled data or data with multimodal distribution were analyzed with the Wilcoxon-Mann-Whitney U test. The chi-square test and Fisher's exact test were used for the analysis of categorically scaled variables. The level of significance for all tests was determined to be a first degree error of 0.05.

The changes between admission day and the day of the final examination were used for the confirmatory statistical analysis of the primary variables. This was carried out according to the distribution of data in the Wilcoxon-Mann-Whitney U tests and in the D test, respectively. In order to keep the experimental error below 5% in multiple testing, the three elementary tests were sequentially compared with the Bonferroni-Holm adjusted threshold, Â/3, C/2, and Â.

Results

Efficacy. Efficacy was excellent. Cerebrolysin®-treated subjects demonstrated marked improvement in all variables.

CGI. In the CGI 91.7% of the subjects in the control group remained unchanged after two weeks (Fig. 1) whereas 88% of Cerebrolysin®-treated patients demonstrated minor improvement. The rest remained unchanged. After four weeks 80% of the subjects in the control group still remained unchanged and 20% showed minor improvement. In

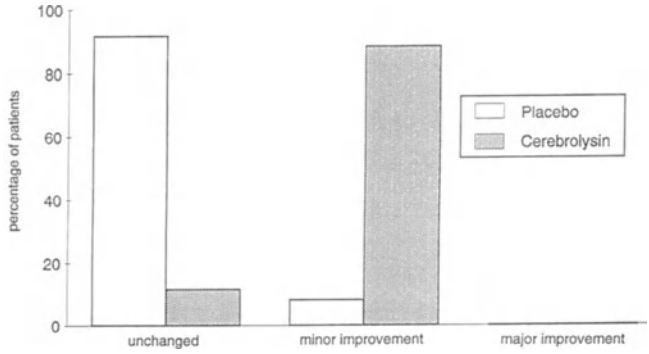

Fig. 1. Effects of 2 weeks (5 days/week) treatment with 30 ml/day Cerebrolysin on clinical impression (CGL item 2) in patients with SDAT versus placebo; 60 Patients/group

contrast, 61.7% of the Cerebrolysin®-treated subjects showed major improvement in the CGI after four weeks (Fig. 2), and the remainder of 38.3% demonstrated minor improvement. The difference was highly significant.

SCAG. The placebo group remained unchanged in SCAG (Fig. 3). Cerebrolysin®-treated patients showed continous improvement during the four weeks, demonstrated a decrease of the SCAG score after two and four weeks.

Trail-making test. The test could not be completed by 13 patients (eight in the Cerebrolysin® group, five in the placebo group). They were excluded. The remaining subjects needed over three minutes to

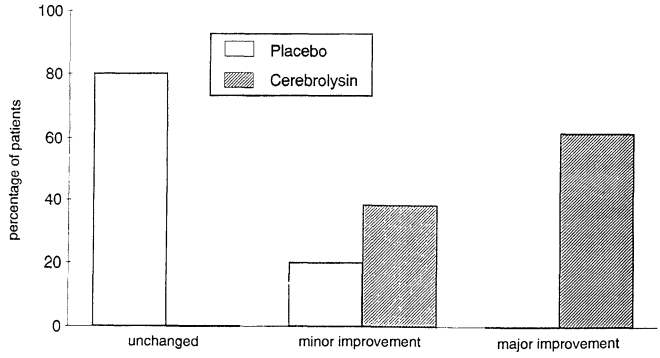

Fig. 2. Effects of 4 weeks (5 days/week) treatment with 30 ml/day Cerebrolysin on clinical impression (CGI item 2) in patients with SDAT versus placebo; 60 Patients/group; $p < 0.0001$

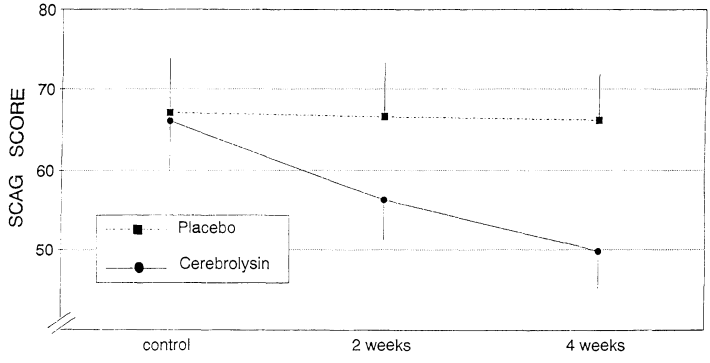

Fig. 3. Effects of 4 weeks (5 days/week) treatment with 30 ml/day Cerebrolysin on improvement in clinical symptomatology (SCAG – SCORE) in patients with SDAT versus placebo. 60 Patients/group; mean +/− sdev.; $p < 0.0001$

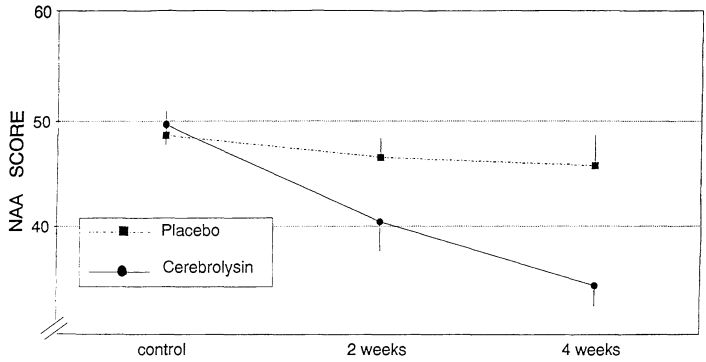

Fig. 4. Effects of 4 weeks (5 days/week) treatment with 30 ml/day Cerebrolysin on activities of daily living (NAA) in patients with SDAT versus placebo. 60 patients/group; mean +/− sdev.; $p < 0{,}0001$

complete the test. The subjects in the Cerebrolysin® group needed about 25 seconds less after four weeks of therapy to complete the test.

Secondary variables. Self-assessment and activities of daily living demonstrated improvement over four weeks (Fig. 4). The placebo group remained virtually unchanged, whereas a substantial improvement was seen in the Cerebrolysin® group in both scales. The decrease of the NAA strongly suggests that the amount of care needed by the patient and the time spent by the caregiver decreased as well.

Side effects. There were no drop-outs or adverse in either group. Daily monitoring of blood pressure, heart rate and blood and urinary chremistries did not reveal any drug-related changes.

Discussion

Cerebrolysin® improved clinical symptomatology and cognitive performance in AD. The improvement was much better than expected. These excellent results suggest that Cerebrolysin® should be used widely in the therapy of AD.

However, some questions remain. The Hamilton Depression Score was not evaluated at the end of the trial, only at the beginning. It would have been interesting to see if the HAMD would have changed in either group. In addition, the placebo group was remarkably stable. This might be due to the short observation period. Although this is a shortcoming of this trial, the improvement seen in this short period is striking.

One major question is the composition of Cerebrolysin®. Although it is known that neurotrophic peptides are present in the drug, it would

be most interesting to sequence the peptides and solve the mechanism of action at the molecular level. These questions can be answered as our knowledge of nerve cells grows in the future.

Earlier trials with Cerebrolysin® confirm the above mentioned clinical results. Although these trials are valid, they cannot be accepted today as pivotal trials because the guidelines have been updated. Even so, these trial constitute an impressive record of Cerebrolysin's® clinical efficacy.

Positive effects in degenerative dementia were found by Hebenstreit et al. (1986). Suchanek-Fröhlich and Wunderlich (1986) examined the effects of Cerebrolysin® versus low-molecular dextrane. There were statistically significant differences in favour of Cerebrolysin® in clinical symptomatology and cognitive performance. Kofler et al. (1989, 1990a,b) examined the nootropic effect of Cerebrolysin® in patients with organic brain syndrome. Statistically significant improvement was seen in self-assessment, cognitive performance, the SCAG scale, the syndrome short test, and the concentration test for the elderly. Vereshchagin et al. (1991) found approxiamately 65% responders to Cerebrolysin® in subjects with vascular dementia (MID). Memory and abstract thinking improved significantly.

The very good results of this trial necessiated a follow-up study in which the same subjects were reassessed six months after the study had finished. Cerebrolysin®-treated patients were significantly superior to the controls (unpublished data). This finding indicates that Cerebrolysin® slows down the degenerative process of AD, most probably due to its neurotrophic activity.

Conclusions

Cerebrolysin® leads to fast and relevant improvement in AD. The short-term activity is excellent. It is of great interest to the public, the medical community, and the families of AD victims to conduct a multicentre long-term trial with Cerebrolysin®. These unexpected but excellent results should be confirmed.

References

Alzheimer A (1907) Über die eigenartige Erkrankung der Hirnrinde. Allg Z Psychiatr 64:146

Alzheimer A (1911) Über eigenartige Krankheitsfälle des späteren Alters. Z Ges Neurol Psychiatr 4:356

Akai F, Hiruma S, Sato T, Iwamoto N, Fujimoto M, Ioku M, Hashimoto S (1992) Neurotrophic factor-like effect of FPF 1070 on septal cholinergic neurons after transections of fimbria-fornix in the rat brain. Histol Histopathol 7:213–221

Albrecht E, Hingel S (1992) The effects of cerebrolysin on the vitality and sprouting of neurons from cerebral hemispheres and from the brain stem of chicken embryos in vitro. Neurobiol Aging 13 [Suppl 1]:S127

Amaduccil, Angst J, Bech P, Benkert O, Bruinvels J, Engel RR, Gottfries CG, Hippius H, Levy R, Lingjaerde O, Lopez-Ibor JJ, Orgogozo JM, Pull Z, Saletu B, Stoll KD, Woggon B (1990) Consensus Conference on the methothology of clinical trials of "Nootropics", Munich, June, 1989. Pharmakopsychiatrie 23:171–175

American Psychiatric Association (1987) Diagnostic and statistical manual of mental disorders, 3rd edn, revised. Washington, DC

Arzneimittelinformation Berlin (1991) Komplikationen mit Todesfolge nach Rinderhirngangliosid GM1 (SYGEN) 1:8

Arzneimittelinformation Berlin (1994) "Aus" für Ginko biloba (TEBONIN)-Ampullen 4:39

Baskys A, Wojtowicz JN (1992) Actions of organ derived preperations on synaptic transmission in the hippocampus. Neurobiol Aging 13[Suppl 1]: 128

Bergerm A (1993) Estimation of concordance rate in demented twin pairs as a function of age of onset and age of sample. Psychiatr Gen 3:139

Breitner J, Gatz M, Betgem Alm, Christian JC, Mortimer JA, Mcclearn GE (1991) Use of twin cohorts for research in Alzheimer's disease. Neurology 43:261–267

Corder E, Saunders AM, Strittmatter WJ, Schmechel DE, Gaskell PC, Small GW, Roses AD, Haines JL, Pericak-Vance MA (1993) Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science 261:921–923

Davidson M, Stern RG, Bierer LM, Horvath TB, Zemishlani Z, Markofsky R, Mohs RC (1991) Cholinergic strategies in the treatment of Alzheimer's disease. Acta Psychiatr Scand 366:47–51

Davis D, Maloney AJ (1976) Selectiv loss of centrocholinergic neurons in Alzheimers' disease. Lancet ii:1403–1405

Davies P (1991) Therapy for Alzheimer's disease: choosing a target. Clin Neuropharmacol 14:124–133

Finali G, Piccirilli M, Oliani C, Piccinin LG (1991) Deprenyl therapy improves verbal memory in amnesic Alzheimer patients. Clin Neuropharmacol 14(6): 523–536

Folstein MF, Folstein SE, Huge BR MC (1975) Mini Mental State. A practical method for grating the cognitive state of patients for the clinician. J Psychiat Res 12:1989–1998

Gauthier S, Gauthier L, Bouchard R, Quirion R, Sultan S (1991) Treatment of Alzheimer's disease: hopes and reality. J Can Sci Neurol 18:439–441

Goate A, Chartier-Harlin MC, Mullan M (1991) Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature 349:704–706

Guy W, Bonato RR (1970) Manual for the Ecdeu assessment battery, 2nd edn, revised. Chevy Chase, Maryland, 12:1–12

Hachinski VC, Iliff LD, Zilhka E, du Boulay GH, Allister VL, Mc Marshall J, Brussel RR, Symon L (1975) Cerebral blood flow in dementia. Arch Neurol 32:632–637

Hamilton M (1960) A rating scale for depression. J Neurol Neurosurg Psychiatry 23:56–62

Hebenstreit GF (1986) Die Wirkung eines Aminosäure-Peptid-Extraktes bei zerebralen Funktionsstörungen in der Gerontopsychiatrie. Neuropsychiatrie 1(1):38–44

Hefti F, Schneider LS (1991) Nerve growth factor and Alzheimer's disease. Clin Neuropharmacol 14:62–76

Hefti F (1983) Alzheimer disease caused by a lack of nerve growth factor? Ann Neurol 13:109–110

Heiss WD, Hebold I, Klinkhammer P, Ziffling P, Szelies B, Pawlik G, Herholz K (1988) Effect of piracetam on cerebral glucose metabolism in Alzheimer's disease as measured by positron emission tomography. J Cereb Blood Flow Metab 8(4):613–617

Hoyer S, Pöhlmann K, Hügel M (1992) Ätiopathogenese vaskulärer und degenerativer Demenzen – aktueller Kenntnisstand. Acta Histochem Su B 42:71–76

Jarvik LF (1991) Calcium channel blocker nimodipine for primary degenerative dementia. Biol Psychiatry 30:1171–1174

Jorm AF, Corten AE, Henderson AS (1987) The prevalence of dementia: a quantitative integration of the literature. Acta Psychiat 76:465–479

Kanowski S, Ladurner G, Maurer K, Oswald WD, Stein U (1990) Empfehlungen zur Evaluierung der Wirksamkeit von Nootropika. Z Gerontopsychol Gerontopsychiatr 3(1):67–79

Kofler B, Erhart C, Erhart P, Harrer G (1989) Zur therapeutischen Wirksamkeit von Cerebrolysin. Psycho 15(8):29–33

Kofler B, Erhart C, Erhart P, Harrer G (1990a) Die Brauchbarkeit der ereigniskorrelierten Negativität zum Nachweis therapeutischer Wirkungen von Nootropika am Beispiel von Cerebrolysin. Z EEG EMG 21:3145–3149

Kofler B, Erhart C, Erhart P, Harrer G (1990b) A multidimensional approach in testing nootripic drug effects (Cerebrolysin). Arch Gerontol Geriatr 10:129–140

Kumar V, Calache M (1991) Treatment of Alzheimer's disease with cholinergic drugs. Int J Clin Pharmacol Ther Toxicol 29:123–137

Mangoni A, Grassi MP, Frattola L, Piolti R, Bassi S, Motta A, Marcone A, Smirne S (1991) Effects of a Mao-B inhibitor in the treatment of Alzheimer disease. Eur Neurol 31:100–107

Mann D MA, Yates PO, Marcyniuk B (1984) Monoaminergic neurotransmitter systems in Alzheimertype dementia and in senile dementia of Alzheimer type. Clin Neuroparhol 3:199–205

Molloy DW, Gordon H, Guyatt DB, Wilson MS, Duke R, Rees L, Singer J (1991) Effect of tetrahydroaminoacridine on cognition, function and behaviour in Alzheimer's disease. Can Med Assoc J 144:129–134

National Institute of Mental Health: Clinical Global Impression (1981, 1986) In: CIPS-Collegium Internationale Psychiatriae Scalarum (eds) Internationale Skalen für Psychiatrie. Beltz Test

Oswald WD, Fleischmann UM (1986) Nürnberger Altersinventar. NAI, Erlangen

Perry EK, Tomlinson BE, Blessed G, Bergman K, Bigson TH, Perry RH (1978) Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. Br Med J 2:1457–1459

Piswanger A, Paier B, Windisch M (1990) Modulation of protein synthesis in a cell-free system from rat brain by cerebrolysin during development and aging. Amino Acids 651–657

Reisberg B, Ferris SH, De Leon MJ, Crook T (1982) An instrument for the assessment of primary degenerative dementia (PDD). Am J Psychiatry 139:1136–1139

Schoenberg B, et al (1987) Alzheimer's disease and other dementing illnesses in a defined United States population: incidence rates and clinical features. Ann Neurol 22:724

Shader R, Harmatz JS, Salzman C (1974) A new scale for clinical assessment on geriatric populations: Sandoz Clinical Assessment-Geriatric (SCAG). J Am Geriatr Soc 22(3):107–113

Shimazu S, Iwamoto N, Itoh T, Akasako A, Seki H, Fujimoto M (1991) Neurotrophic activity of cerebrolysin. Second International Springfield Symposium on Advantage in Alzheimer Therapy, Springfield, USA

Shimazu S. Tachikawa N, Iwamoto N, Itoh D, Komatsu S, Fujimoto M (1992) The neurotrophic and brain protective effect of cerebrolysin. Neurobiol Aging 13[Suppl 1]:50

Skoog I, Nilsson L, Palmertz B, Andreasson LA, Svanberg A (1993) A population based study of senile dementia in 85 year olds. N Engl J Med 328:153–158

Suchanek-Fröhlich H, Wunderlich E (1986) Über die Wirksamkeit eines Aminosäure-Peptid-Extraktes – Randomisierte Doppelblind-Placebo-Vergleichsstudie. Neuropsychiatrie 1(1):45–48

Svennerholm L, Gottfries CG, Blennow K, Fredman P, Karlsson I, Mansson JE, Toffano G, Wallin A (1990) Parenteral administration of GM1 ganglioside to presenile Alzheimer patients. Acta Neurol Scand 81:48–53

Travis J (1993) New piece in Alzheimer's puzzle. Science 261:828–829

Tuszynski MH, Gage FH (1990) Potential use of neurotrophic agents in the treatment of neurodegenerative disorders. Acta Neurobiol Ex 50:323–332

Vereshchagin N, Lebedeva NV, Suslina ZA, Solovyov OI, Priadov MA, Altunina MN (1991) Mild forms of multiinfarct dementia: effectiviness of cerebrolysin. Sowj Med (Moskau) 11:6–8

Windisch M, Piswanger A (1987) Einfluß einer 7tägigen Behandlung mit Cerebrolysin auf die Atmung von Hirnmitochondrien. Neuropsychiatrie 1(2):83–88

Zerssen D von, Möller H-J (1980) Psychopathometrische Verfahren in der psychiatrischen Therapieforschung. In: Bifang S (ed) Evaluations-Forschung in der Psychiatrie: Fragestellung und Methoden. Enke, Stuttgart.

Correspondence: Prof. Dr. E. Rüther, Psychiatrische Universität sklinik, Georg-August Universität, v. Sieboldstrasse 5, D-37075 Göttingen, Federal Republic of Germany

Subject Index

P. Riederer, M.B.H. Youdim (eds.)

Iron in Central Nervous System Disorders

1993. 50 figures. VII, 205 pages.
Soft cover DM 98,–, öS 686,–
ISBN 3-211-82520-7
(Key Topics in Brain Research)

T. Nagatsu, H. Narabayashi, M. Yoshida (eds.)

Parkinson's Disease. From Clinical Aspects to Molecular Basis

1991. 52 figures. VIII, 220 pages.
Soft cover DM 98,–, öS 686,–
ISBN 3-211-82272-0
(Key Topics in Brain Research)

Springer-Verlag Wien New York
Sachsenplatz 4–6, P.O.Box 89, A-1201 Wien · 175 Fifth Avenue, New York, NY 10010, USA
Heidelberger Platz 3, D-14197 Berlin · 3-13, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan

K. Maurer, P. Riederer, H. Beckmann (eds.)

Alzheimer's Disease. Epidemiology, Neuropathology, Neurochemistry, and Clinics

1990. 118 figures (9 in color). XIX, 581 pages.
Soft cover DM 176,–, öS 1230,–
ISBN 3-211-82197-X
(Key Topics in Brain Research)

H. Przuntek, P. Riederer (eds.)

Early Diagnosis and Preventive Therapy in Parkinson's Disease

1989. 59 figures (1 in color). XIV, 442 pages.
Soft cover DM 135,–, öS 950,–
ISBN 3-211-82080-9
(Key Topics in Brain Research)

Prices are subject to change without notice

Springer-Verlag Wien New York
Sachsenplatz 4–6, P.O.Box 89, A-1201 Wien · 175 Fifth Avenue, New York, NY 10010, USA
Heidelberger Platz 3, D-14197 Berlin · 3-13, Hongo 3-chome, Bunkyo-ku, Tokyo 113, Japan

Springer-Verlag and the Environment

WE AT SPRINGER-VERLAG FIRMLY BELIEVE THAT AN international science publisher has a special obligation to the environment, and our corporate policies consistently reflect this conviction.

WE ALSO EXPECT OUR BUSINESS PARTNERS – PRINTERS, paper mills, packaging manufacturers, etc. – to commit themselves to using environmentally friendly materials and production processes.

THE PAPER IN THIS BOOK IS MADE FROM NO-CHLORINE pulp and is acid free, in conformance with international standards for paper permanency.