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The 4th Hungarian Conference on Alzheimer’s Disease and Related Disorders was held in Szeged, Hungary, on October 7–9, 1998. Approximately 150 participants representing research workers from 13 different countries took part, together with industrial experts, hospital practitioners, and family doctors. The conference language was English and the reports are published in a supplement to Clinical Neuroscience (Idegtudományi Szemle) (Vol. 51, pp. 1–64, 1998).

OPENING LECTURE

E. Giacobini (Geneva Medical School, Geneva, Switzerland) delivered the opening lecture, dealing with the therapy of Alzheimer’s disease (AD), present and future. He concluded that at present the cholinesterase inhibitors are the drugs of choice for the treatment of AD. Following a first generation of non-specific drugs such as physostigmine, a second generation of more selective products has been developed, with less severe side effects at effective doses. Recent clinical results on the effect of drugs on cognition and on behavioral symptoms in people with AD confirm early predictions of behavioral pharmacology from studies in animals and humans. Cholinesterase inhibitors, however, are not the only agents related to cholinergic therapy. As discussed during the conference, muscarinic and nicotinic agonists and antagonists are under development. Giacobini called attention to potentially interesting combinations of cholinesterase inhibitors with estrogens, anti-inflammatory drugs, and anti-oxidants. Other drugs based on amyloid precursor protein (APP), amyloid-β (Aβ), apolipoprotein E (ApoE) and presenilins are still at the preclinical stage of investigation.

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PLENARY LECTURES

C. Geula (Harvard Medical School, Boston, USA) surveyed the earlier results on the pathogenesis of AD. He elegantly demonstrated the neuropathological hallmarks (the senile plaques [SPs] and neurofibrillary tangles [NFTs]) of AD. The SPs are composed primarily of extracellularly accumulated A\textbeta protein, which is a part of the much larger APP and must be cleaved out of the parent protein through proteolytic processing. The NFTs are intracellular accumulations of neurofibrillar elements within the cytoplasm. A major component of the NFTs is the abnormally phosphorylated tau. A third pathological hallmark of AD is the neuropil threads. Consistent and widespread degeneration of cholinergic axons originating in the basal forebrain can likewise be revealed. The most severe cortical cholinergic denervation in AD is found in cortical zones within the temporal lobe. On the other hand, the primary sensory and motor cortical regions are, in general, relatively free of AD pathology. Geula pointed out that that SPs and NFTs may first appear many years, if not decades, before the clinical manifestation of dementia.

Using an animal model of nonhuman primate, Geula injected small quantities of A\textbeta (approximately equal to the amount as can be found in a single human SP) into the cerebral cortex, and demonstrated the extensive loss of neurons and the hyperphosphorylation of tau. With this animal model, Geula was able to produce most of the pathology observed in the AD brain. His results support the suggestion that SP formation may represent an early pathological event, which may contribute to the formation of NFT. He also pointed out that, although it is generally believed that cortical cholinergic denervation in AD is the result of a cascade of neuropathological events, his findings indicate that cholinergic abnormalities may contribute directly to the pathological process.

A. Fisher (Israel Institute for Biological Research, Ness-Ziona, Israel) summarized his results with a number of m\textsubscript{1} selective muscarinic agonists [AF102B, AF150(S), and YM79] designated for the treatment of AD. In addition, he reported the effects of the nonselective muscarinic agonist, milameline, and the partial m\textsubscript{1} agonists SB-202026 and LU 25-109. It was shown that the m\textsubscript{1}-selective agonist AF102B and talsaclidine increased the cleavage of APP, producing non-amyloidogenic APP (APPs) in rat cortical brain slices. On the other hand, AF102B and AF150(S) enhanced APP release in rat primary cell cultures of the hippocampus and cerebral cortex. In PC12M1 cells, AF102B and AF150(S) increased APP secretion, this effect being further augmented by neurotrophins such as nerve growth factor (NGF) and basic fibroblast growth factor (bFGF). He has also shown that, via the activation of m\textsubscript{1} mAChR, the AF series, synergize with NGF and promote the survival of cultured primary CNS neurons.

Most of the reported m\textsubscript{1}-selective agonists were evaluated in a variety of animal models for AD in which AF102B and AF150(S) restored memory and learning deficits. In Apo-E-deficient mice, AF150(S) restored to normal the impairments of working memory, ChAT and AChE activities, the m\textsubscript{1} mAChR level, and tau protein phosphorylation. With this result, Fisher demonstrated a link between the m\textsubscript{1} mAChR signal transduction system(s) and the neuronal cytoskeleton via regulation of the phosphorylation of tau microtubule-associated protein. This results might provide a novel therapeutic strategy in AD based on m\textsubscript{1} agonists via impairment of paired helical filament formation by decreasing tau phosphorylation.

P. G. M. Luiten (Univ. Groningen, The Netherlands) reported experimental and post-mortem human data on some of the risk factors that play key roles in the cascade leading to neuronal and behavioral dysfunctions in aging and dementia. He pointed out the impor-
tance of the microvascular integrity (the anatomical substrate of the blood-brain barrier) in normal brain functions. By means of ultrastructural studies, he demonstrated an almost linear progression in the deterioration of the microvascular wall in the cortex of aged rats. The basement membrane was thickened, collagen had accumulated in the basement membrane, and the pericytes around the vascular bed were degenerated. The process of capillary degeneration was potentially delayed by chronic treatment with calcium antagonists, which indicated that the degeneration process was calcium-dependent. Luiten has revealed a similar pattern of microvascular degeneration in the postmortem human brain of aged humans without neurological history and in the brains of dementia patients. In autopsy material from patients with various types of dementia, the deposits in the capillary bed were more than double those in aged matched controls.

AMYLOID AND ALZHEIMER’S DISEASE

K. Beyreuther (Univ. Heidelberg, Germany) discussed the physiological functions of AD-related genes and the βA4 amyloid peptide. By studying the intraaxonal transport of APP, Beyreuther has discovered that the axonal transport is dependent on the βA4 domain of APP. It has also been shown that cholesterol depletion inhibits βA4 generation and that the regulation of cholesterol homeostasis by ApoE modulates neuronal APP transport and processing.

B. Penke (Albert Szent-Györgyi Medical Univ., Szeged, Hungary) dealt with the role of the aggregation of βA peptide in AD and neurotoxicity. He presented data on the aggregation and disaggregation processes of the peptides, demonstrating that dimethyl sulfoxide disaggregated all the aggregated βA peptide and that the aggregation tendency is highly sequence dependent.

M. Hollósi (Eötvös Loránd Univ., Budapest, Hungary) discussed the racemization-induced defolding and aggregation of βA fragment, in which process metal ions, and especially Al³⁺, may play a catalytic role.

I. Degrell (Univ. Debrecen, Hungary) presented evidence that the composition of the cerebrospinal fluid is different in patients with various dementias (presenile dementia of Alzheimer type [PDAT], senile dementia of Alzheimer type [SDAT], and multiinfarct dementia [MID]). He established that the glucose concentrations were significantly lower in PDAT than in SDAT, MID, and the reference group. Lactate levels were also lower in PDAT than in SDAT and MID, but there was no significant difference compared with the reference group. Pyruvate levels were significantly lower in MID compared with the reference group. Xanthine levels in the various dementia groups did not display any differences between the different types of dementias. He demonstrated that the levels of amino acids may be characteristic of the type of dementia. It was suggested that an active transport process is responsible for carrying amino acids through the blood-brain barrier.

CHOLINESTERASE IN ALZHEIMER’S DISEASE

B. H. Schmidt (Bayer CNS Research, Cologne, Germany) discussed the significance of drugs to reconstitute the synaptic levels of acetylcholine (ACh) in the brain of AD patients. At present the cholinesterase inhibitors are clinically the most advanced therapeutic...
drugs. Various types (carbamate, organophosphorus, aminoacridine, benzylpiperidine, and sulfonyl halogenide derivatives) of cholinesterase inhibitors have been tested. A 70% inhibition of acetylcholinesterase (AChE) would be required for therapeutic efficacy, but achievement of this high inhibition of the enzyme is not well tolerated and may have side effects. The differing tolerability profiles of cholinesterase inhibitors should therefore be determined by pharmacodynamic and pharmacokinetic means.

N. C. Inestrosa (Catholic Univ. Chile, Santiago) reported that AChE is able to promote the formation of amyloid fibrils and hypothesized that the fibrils of the AChE-Ab complexes may be neurotoxic. His hypothesis was underscored with different experiments which indicated that AChE may increase the neurotoxicity of Ab.

Z. Rakonczay (Albert Szent-Györgyi Medical Univ., Szeged, Hungary) presented his results on the search for selective inhibitors for the different molecular forms (G1 and G4) of AChE. He tested various AChE inhibitors (tacrine, bis-tacrine, heptyl-physostigmine, TAK-147, and metrifonate) in the human striatum of a normal brain sample. Among the inhibitors tested, only heptyl-physostigmine displayed a preferential inhibition of the G1 form.

**ANIMAL MODELS**

I. Hanin (Loyola Univ., Chicago, IL, USA) described various animal models introduced to study cholinergic dysfunctions in AD. In his review, he summarized the known neurochemical, neuropathological and behavioral effects, and the possible mechanism of action of AF64A. He compared and contrasted these effects with those of the excitotoxins and 192IgG-saporin and with observations in AD. He demonstrated that the ChAT, AChE activities, high affinity choline transport HACHT, and the memory dysfunction parallel those found in AD.

M. Barcikowska (Polish Academy of Science, Warsaw) demonstrated that in ischemic brain (evoked by cardiac arrest) the injection of synthetic human Ab peptide results in extravasation and the deposition of diffuse plaques in the brain parenchyma. Animals receiving Ab for 3 m exhibited diffuse plaques and the neuronal, glial, ependymal, endothelial, and pericyte cell bodies were filled with Ab. No such deposition of Ab was observed after 1 y, suggesting that the artificial amyloid diffuse plaques were not stable and could be cleared slowly from the brain parenchyma. Barcikowska emphasized the importance of the intact blood-brain barrier in the defense mechanism against circulating Ab.

P. Klivényi (Albert Szent-Györgyi Medical Univ., Szeged, Hungary) considered the importance of a genetic animal model to study the role of reactive oxygen species under physiological and pathological conditions.

N. Bons (Univ. Montpellier II, France) demonstrated that the lemurian primate Microcebus murinus is a convenient natural animal model to study the neuropathological, neurochemical, and behavioral alterations found in AD.

**NEW RESULTS IN ALZHEIMER’S DISEASE RESEARCH**

G. Perry (Case Western Reserve Univ., Cleveland, USA) discussed the role of mitochondrial abnormalities in AD. He demonstrated that the mitochondria in hippocampal
neurons in AD display a 2 to 3-fold proliferation, specifically for large neurons, compared with age-matched controls.

D. Kovacs (Harvard Medical School, Boston, MA, USA) discussed the role of presenilin (PS1 and PS2) mutation in AD. She found that the PS2-N14I FAD mutant increases the caspase cleavage of PS2 by roughly 3-fold in inducible H4 cell lines, and furthermore that presenilin mutants may affect cellular caspases.

M. Novak (Slovak Academy of Sciences, Bratislava, Slovak Republic) discussed the role of presenilin (PS1 and PS2) mutation in AD. She found that the PS2-N14I FAD mutant increases the caspase cleavage of PS2 by roughly 3-fold in inducible H4 cell lines, and furthermore that presenilin mutants may affect cellular caspases.

M. Novak (Slovak Academy of Sciences, Bratislava, Slovak Republic) presented data on the truncation of tau and neurodegeneration. He emphasized that the mechanism by which tau protein is modified to take part in the neurofibrillary pathology in AD is unknown. He suggested that there should be at least one step between the phosphorylation of tau and cell death. His results suggested that the truncation of tau precedes the cytoskeletal changes related to the neurofibrillary pathology. He showed that tau is an in vivo substrate for proteases during apoptosis, and that the truncation of tau is not only an early, but also, together with hyperphosphorylation, the most deleterious event in neuronal degeneration.

R. G. Wiley (Vanderbilt Univ., Nashville, USA) put forward experimental evidence for the selective lesioning of cholinergic neurons expressing the low-affinity nerve growth factor receptor, p75, particularly in the cholinergic basal forebrain (CBF), with the immunotoxin 192IgG-saporin. He demonstrated that 4 μg of this immunotoxin injected intravenously causes complete or near-complete destruction of CBF and deficits in behavior in several tasks, including passive avoidance, spatial tasks and attentional tasks.

A. Matsumoto (Kobe Univ., Kobe, Japan) discussed the abnormal deposition and fibril formation of Aβ in AD brains, which process is crucial for understanding advances in AD therapy. His results demonstrate that in the human hippocampus a 40-kDa protease can be found which has the unique property of cleaving natural APP and directly generating Aβ-containing C-terminal peptides at physiological pH.

A. Wevers (Univ. Cologne, Germany) investigated the expression of two nAChR subunits (α4 and α7) on the transcriptional and translational levels in control and AD samples. The in situ hybridization experiments revealed no alterations in the densities of α4 and α7 mRNA-expressing neurons in the cortex of AD patients, but at the protein level a marked decrease was evident. She suggested a distinct decrease at the protein level in the α4 and α7 subunits of the nAChR.

**AMYLOID-β PEPTIDES**

S. Kar (McGill Univ., Montreal, Canada) revealed that soluble Aβ1-28, Aβ1-40, Aβ1-42, and Aβ25-35 inhibited K+-evoked ACh release from rat hippocampal slices. The inhibitory effect was observed in the frontal cortex, but not in the striatum.

P. Kasa (Albert Szent-Györgyi Medical Univ., Szeged, Hungary) demonstrated that Aβ1-42, Aβ25-35, Aβ31-35, and Aβ34-39 are toxic to cholinergic and AChE-positive neurons in in vitro tissue cultures. He observed that Aβ dislocated the AChE reaction end-product from the perinuclear site to the perikaryonal membrane. Using quantitative methods, reductions in the numbers of AChE-positive and vesicular acetylcholine transporter-positive neurons were revealed. In neuropharmacological experiments, the ACh pool present in...
cholinergic neurons was diminished in the treated tissue cultures. He presented evidence that the aggregation of Aβ is not a prerequisite for the neurotoxic effect of the peptide.

G. Jancsó (Albert Szent-Györgyi Medical Univ., Szeged, Hungary) found that Aβ_1–42 impairs the blood-brain barrier after intracarotid infusion of the peptide in rats.

G. Laskay (József Attila Univ., Szeged, Hungary) presented results relating to the use of Aβ_1–42. He demonstrated that the peptide induces hyperpolarization in M213-20 cells, an immortalized rat striatal cell line.

C. Abraham (Boston Univ., Boston, MA, USA) identified in the AD brain a candidate β-secretase, a cysteine protease termed bleomycin hydrolase.

A. Mitro (Slovak Academy of Sciences, Bratislava, Slovak Republic) demonstrated a monoclonal antibody (MN5) against a fragment of Aβ that stains phosphorylated tau protein in AD brain samples. He showed that MN5 does not recognize the recombinant form of tau and adult tau isoforms.

T. Harkány (Haynal Imre Univ., Budapest, Hungary) presented results indicating that MK-801 and vitamins E and C have neuroprotective effects on Aβ_1–42 neurotoxicity.

B. Csillik (Bay Zoltán Institute for Biotechnology, Szeged, Hungary) demonstrated that the number of cholinergic principal cells is 512 ± 25 per mm³ in the nucleus basalis of Meynert in the basal forebrain of primates, which decreases to 160 ± 21.6 per mm³ in old (>30 y) animals.

D. Budai (Juhász Gyula College, Szeged, Hungary) reported the effects of Aβ_1–40 and Aβ_25–35 in extracellular recordings from dorsal spinal cord horn neurons. He has observed that, in the presence of Aβ_1–40, a significant decrease in the kainic acid-evoked response can be obtained. In contrast, an increase occurred in the NMDA-evoked responses. He concluded that Aβ is capable of influencing neurotransmission by EAA.

M. Páékáski (Albert Szent-Györgyi Medical Univ., Szeged, Hungary) presented morphological and semiquantitative (immunoblot) data indicating that the levels of APP can be modulated by mechanical damage and by the muscarinic receptor antagonist scopolamine. Her experiments support the suggestions that cholinergic function can influence APP biosynthesis.

THE ROLE OF GLIAL CELLS IN ALZHEIMER’S DISEASE

L. Latzkovits (Albert Szent-Györgyi Medical Univ., Szeged, Hungary) established the role of glial cells in the pathomechanism in AD and related disorders. He revealed that activation of the astroglia by immunological factors in neuronal-glial interaction may be a component resulting in neurodegeneration.

G. Laskay (József Attila Univ., Szeged, Hungary) presented experimental data that the effects of the Aβ-induced long-term elevation of [Ca^{2+}] in rat astrocytes can be prevented by a putative antagonist Pr-Ile-Ile-Gly-Leu-NH₂.

C. Torday (Albert Szent-Györgyi Medical Univ., Szeged, Hungary) examined the effectiveness of the astrocyte defense system in different types of free radical insults both in the absence and in the presence of various scavengers. The luminol-amplified chemoluminescence method was applied to the in situ monitoring of different free radicals generated in primary rat astroglial cell cultures.
TRACE METALS AND ALZHEIMER’S DISEASE

E. Andrási (Eötvös Loránd Univ., Budapest, Hungary) applied inductively coupled plasma atomic emission spectrometry (ICP-AES) to demonstrate quantitatively that there are significant decreases in boron and phosphorus, but an increase in sulfur concentration in AD samples as compared to control samples.

T. Harkány (Haynal Imre Univ., Budapest, Hungary) used a rat model and demonstrated a neuroprotective effect of a complex containing trace metals and vitamins against anoxia-induced free radical generation.

The scientific presentations were followed by lively discussions and open debates on the issues. Participants found the organization and scientific standards to be high with a very instructive program.