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Reviews

Alzheimer's disease: fundamental and therapeutic aspects

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Abstract. Alzheimer's disease is the most common type of progressive and debilitating dementia affecting aged people. In some early - as well as late-onset familial cases, a genetic linkage with chromosomes 14, 21 (early-onset) or 19 (late-onset) has been indicated. Furthermore, a direct or indirect role has been attributed to normal or structurally altered amyloid β-protein (concentrated in senile plaques) and/or excessively phosphorylated tau protein (located in neurofibrillary tangles). Degeneration of cholinergic neurons and concomitant impairment of cortical and hippocampal neurotransmission lead to cognitive and memory deficits. Several compounds are being tested in attempts to prevent and/or cure Alzheimer's disease, including tacrine, which has very modest efficacy in a sub-group of patients, and new acetylcholinesterase inhibitors. Pilot experiments have also been launched using nerve growth factor (NGF) to prevent or stabilize the processes of cholinergic pathway degeneration. Alternatively, antioxidants, free radical scavengers and/or non steroidal anti-inflammatory agents may be screened as potential therapies for neurodegenerative diseases induced by multiple endogenous and/or exogenous factors. The recent use of transgenic mice, in parallel with other genetic, biochemical and neurobiological systems, in vivo and/or in vitro (cell cultures), should accelerate the discovery and development of specific drugs for the treatment of Alzheimer's disease.
Key words. Alzheimer's disease; chromosomes 14, 19, 21; amyloid β-protein; spirochetes; tau protein; choline transporter; cholinergic neurons; acetylcholinesterase inhibitors; tacrine; antioxidants; free radicals; nerve growth factor (NGF); indomethacin; apoptosis; nitric oxide.

Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease of old age and affects millions of people over the age of 65 in the western world. AD is accompanied by the gradual and progressive loss of functional and psychomotor abilities. In the final stage of the illness, patients are severely demented and loss of ambulation occurs, accompanied by grasping and sucking reflexes[17]. Unfortunately, at present there is practically no effective treatment to prevent or slow the progression of this distressing neurodegenerative illness[8].

Epidemiological and genetic studies of AD

It has long been thought that AD could be caused by many different factors, both environmental and genetic. This belief has been supported by linkage studies which indicate aetiological heterogeneity (ref. 22 and references therein). Considerable progress has been made in determining the identity of the proteins and molecular pathology involved in AD, the most widely known of which are the inevitability of AD in Down's syndrome patients with trisomy of chromosome 21, and the point mutations of the β-amyloid precursor protein gene on chromosome 21 (ref. 25).

Genetic mutations responsible for early-onset familial forms of AD may also affect chromosome 14 (ref. 52). In contrast, mutations on chromosome 19 and the concomitant expression of variant apolipoprotein E4 (ApoE4) from ApoE gene (ε4 allele) are associated with sporadic and late-onset familial forms of AD[76]. Again, the most plausible hypothetical link between ApoE4 and AD involves the β-amyloid cascade, since a colocalization of ApoE4 and amyloid-β-protein (β/A4) was found in the brains of AD patients[53]. Furthermore, the ε4 allele was shown to be strongly connected with increased vascular and plaque β/A4 deposition in brains of patients who died with late-onset AD[66]. However, these genetic causes of AD account for a small proportion of patients who suffer from the disease (less than 1%). It is probable that the overwhelming majority of cases is caused by a variety of environmental factors which may be either sufficient to trigger disease by themselves, or sufficient when acting synergistically with the patient genotype[65]. In this context, it is worth noting that previous research into the causes of AD has pointed to a possible impairment of glucose transport[30]. This metabolic abnormality and its putative impact on cellular and molecular mechanisms of the sporadic type of AD dementia have been reviewed recently[34].

Senile plaques and amyloid β-protein: direct or indirect link with AD?

Despite the apparent aetiological heterogeneity of AD, few topics in neuroscience have generated more interest

in recent years than the role attributed to the β/A4 and its much larger precursor protein (βAPP)[1,37,68,69]. Although a direct neurotoxic effect of β/A4 in vivo and in vitro is still a matter of hot controversy[47], the deposition of β/A4 in the brain (concentrated in the senile plaques) and its microvasculature is an invariable feature of AD that appears to precede the onset of dementia by many years. However, a strict correlation between β/A4 deposition, which can occur in various brain regions, and neurodegenerative events is far from unequivocally demonstrated. Furthermore, a substantial β/A4 deposit can be observed in the brains of many elderly individuals, who are not at all affected by cognitive deficiencies. Nevertheless, it had been assumed that the proteolytic release of β/A4 from the transmembrane region of βAPP was an aberrant event, requiring prior membrane injury. However, it has recently been shown that β/A4 is continuously secreted from healthy neural and non-neural cells in culture and circulates in human CSF and blood[27]. This finding has led to many studies of the dynamics of β/A4 formation and clearance in health and in genetic forms of AD. A potential therapeutic targeting of AD at this level has been also envisaged[18,59,69]. A recent communication has shown that activation of the protein kinase C signal transduction pathway down-regulates the generation of the amyloidogenic β/A4 peptide[35]. Pharmacological agents that activate this system, including a variety of first messengers, could theoretically slow the development or growth of some β/A4 plaques during the early stages of AD[35].

Surprisingly, a first report by Miklossy[49] has shown that spirochetes can be observed in blood, cerebrospinal fluid and brain from AD patients, in addition to a positive immunoreaction with monoclonal antibodies against the amyloid β-protein precursor. These results would suggest a causal relationship between spirochetes and AD, the parasites being the source of the β/A4 deposits in the brain of patients. This possible relationship was debated in an accompanying editorial comment[29]. A further ultrastructural examination of the identity of the infectious microorganisms has strengthened the first observation[50]. However, the new arguments are exclusively morphometric, leaving many puzzling questions unsettled[29].

Neurofibrillary tangles and the tau protein connection

Detection of numerous neurofibrillary tangles (NFT) in the brain of patients affected by AD is another common, and apparently more significant, pathological feature of the illness[26,37,45]. Electron microscopy shows this fibrillary material to be made of paired helical filaments (PHF), which, in turn, are derived from microtubule-associated proteins (MAP). Tau protein, a constituent of normal brain, appears to be the main component of PHF[42,54]. In AD, abnormally phosphorylated insoluble forms of tau (e.g. tau protein A68) are involved in the formation of PHF and are responsible for the major structural and functional changes[74]. Thus, PHF may result from excessive phosphorylation of normal tau, which in turn could interfere with the ability of tau to stabilize microtubules, leading to disruption of neuronal transport and reducing cell viability. A protein kinase that phosphorylates PHF proteins in vitro has been identified in brain tissue preparations from patients with AD[77]. Attempts to block the aberrant phosphorylation of tau proteins could have therapeutic potential.

Cholinergic neurons: possible pharmacological targeting

Based on other fundamental features of AD pathology, another possible therapeutic avenue is a reactivation of the cholinergic system[62]. This strategy is based on the hypothesis that AD is associated with decreased central cholinergic transmission that contributes significantly to the cognitive impairment associated with this disorder[9]. Several observations favor such a hypothesis. For example, there are large reductions in the activity of choline acetyltransferase (CAT) in the cortex and the hippocampus of brains of patients with AD, and this is believed to be due to the degeneration of cholinergic neurons originating in the nucleus basalis of Meynert. There is also an apparent correlation between the reduced CAT activity and the severity of the dementia measured by cognitive and behavioral rating scales. Furthermore, muscarinic antagonists such as scopolamine impair cognitive performance in normal subjects, whereas in animals, muscarinic agonists have been found to reverse the effects of cholinergic lesions[9,62]. Therefore, the cholinergic hypothesis predicts that potentiation of central cholinergic function should ameliorate the cognitive impairment associated with AD.

Another abnormality of the cholinergic system was recently found in the brains of patients affected by AD[72]. Unexpectedly, the high affinity choline transporter in the cortical regions was found to be overexpressed, due to a possible acceleration in the frequency of firing of surviving neurons. This adaptive reaction would result from the loss of cholinergic neurons and concomitant impairment of neurotransmission that occur in the disease. Since the acceleration of firing may be detrimental to the cholinergic cells, it was proposed that drugs (e.g. muscarinic agonists) which would decrease neuronal firing could restore cholinergic transmission[72].

Unlike the pharmacological targeting of cholinergic neurons, a strategy aimed at the modulation of monoamine neurotransmitters (e.g. serotonin, dopamine and noradrenaline) does not appear to be supported by experimental data. Studies relating these neurotransmitters to the symptomatology of AD indi-

cate that their dysfunction is more closely linked to normal aging and/or to non-cognitive than to cognitive changes. This topic has been reviewed recently[57].

The substitution therapy of AD: efficacy of acetylcholinesterase inhibitors?

This therapy is based upon the simple premise that if the cognitive deficits are due to the loss of cholinergic activity, then enhancing this activity with choline supplementation (lecithins) and/or acetylcholinesterase inhibitors might attenuate the deficits. While this strategy has proved useful in the symptomatic treatment of Parkinson's disease with L-dopa, it has not been really effective in AD[78]. Most multicenter trials aimed at the reactivation of cholinergic pathways have been performed with tacrine (tetrahydroaminoacridine, Cognex®). This potent, centrally active and reversible acetylcholinesterase inhibitor was first available in Australia in the mid 1940s, when it was used together with morphine to lessen respiratory depression without affecting analgesia. Tacrine has been used alone, or in combination with lecithin, to treat AD[6,13,14,16,21]. None of the larger, better-controlled studies confirmed the dramatic clinical efficacy of an earlier trial performed with only 17 patients[75]. At best, some showed a slight but statistically significant improvement in one parameter[6,21]. Only a small percentage of patients, moderately affected or treated at an early stage of AD, seem to benefit from the drug[16].

In effective doses (80 to 160 mg/day) tacrine has several peripheral side-effects (mainly gastrointestinal troubles resulting from cholinergic activation), although these are mild and transient. A dose-dependent and normally reversible hepatotoxicity has also been detected in most clinical studies, including a recent multicenter clinical trial applied to 2446 men and women[79].

Velnacrine, a 1-hydroxy-derivative of tacrine, was developed to lessen the hepatotoxicity by facilitating glucuronidation and elimination[70]. Its efficacy as well as its tolerability are still to be evaluated on a larger number of patients for prolonged periods of time[24]. Other acetylcholinesterase inhibitors have been proposed and/or tested as AD treatments[40]. These include the earliest described, physostigmine and pyridostigmine. The former has too many peripheral and central side-effects, while its derivative epastigmine (heptylphysostigmine, MF 201) has been recalled due to haematological problems[40]. The second does not cross the blood-brain barrier, and thus it does not significantly improve cognitive function in AD patients[51]. Among other new inhibitors presently under investigation are galanthamine (a tertiary amine of the phenanthrene group) and epigalanthamine; huperzine A, an alkaloid extracted from a Chinese club moss (*Huperzia serrata*); metrifonate (trichlorfon), a long-lasting inhibitor used for the treatment of schistosomiasis; SDZ ENA 713, a centrally-active, selective and long-lasting inhibitor, which enters into the CNS after parenteral and oral administration; and SM-10888 (9-amino-8-fluoro-1,2,34-tetrahydro-2,4-methanoacridine citrate) that is almost equipotent with tacrine at inhibiting acetylcholinesterase activity. A comparative survey of the pharmacological and possible therapeutic properties of these agents has been published recently[40]. The list of additional centrally active inhibitors includes NIK-247 (9-amino-2,3,5,6,7,8-hexahydro-1H-cyclopenta(b)-quinoline monohydrate hydrochloride), a 4-aminopyridine derivative and MDL 73745 (ref. 23, 82).

Neurotrophic factors enter the clinic

Attempts to treat AD with the neurotrophin NGF (Nerve Growth Factor), which could counter the degeneration of cholinergic neurons projecting to the hippocampus, a recognised memory center, were recently initiated[41]. Keeping the cholinergic neurons alive would then be another possible AD therapy. A preliminary study performed in Sweden has shown that infusions of NGF into the brain of a patient can improve performance in a memory test[56]. Other similar trials have been launched in the USA[2]. The investigators do not expect NGF to cure the disease; the hope is that NGF treatment would have, at best, a significant behavioral effect. Other neurotrophins, neuronal growth factors or insulin-like growth factors are under investigation for other neurological diseases where current therapies are virtually non-existent[2].

Alternative therapies of AD

A heterogenous collection of natural or synthetic agents has been tested and/or proposed for the treatment of AD[12]. Many vasodilator drugs are used in peripheral as well as cerebral arterial diseases including the various forms of dementia[10,44]. Nevertheless, the apparent vasodilatory action of these drugs is far from beneficial in all cases. Some other drugs having no direct vasodilatory action were also prescribed for similar pathological conditions. These include nootropic drugs (e.g. piracetam and analogs, ergot alkaloids, Ginkgo biloba, etc.) as well as non specific (cinnarizine and flunarizine) and specific (nimodipine) calcium channel antagonists. As a result, the term of 'cerebroactive' drugs was coined to designate a new heterogenous family of agents used in the treatment of cerebral insufficiencies (Age Related Mental Diseases), as well as vascular dementia (Multiple Infarct Dementia) and presenile or senile dementia of AD type[73]. Pharmacological specificity (using appropriate in vivo and in vitro models) and clinical indications remain to be re-evaluated for every compound except nimodipine/Nimotop® (prevention of

cerebral ischaemia following sub-arachnoid haemorrhages). No such drug has yet been proven to be useful in the treatment of AD, although one (co-dergocrine/Hydergine® that is very popular in European countries) has been authorized by the FDA for 'idiopathic decline in mental capacity', a condition that may accompany the aging process or the various forms of dementia.

A new therapeutic approach has recently been undertaken with the 6-month, double-blind, placebo-controlled administration of 100 to 150 mg/d indomethacin to protect mildly-to-moderately impaired AD patients from the degree of cognitive decline expected in a matched, placebo-treated group[63]. The rationale of this therapy derives from the findings that in the brain of AD patients, numerous acute phase reactants and immune-related markers are present, very often in association with amyloid-β-protein deposits[63]. If it can cross the blood-brain barrier, an appropriate non-steroidal anti-inflammatory drug (NSAID) should be able to inhibit the cascade of immuno- and inflammatory-reactions that initiates and/or follows the course of AD. The therapeutic potential of indomethacin and other NSAIDs for the treatment of this neurological illness has recently been discussed[64]. Since larger clinical trials are presently underway, the results are eagerly expected.

A place for antioxidants and/or free radical scavengers?

It is worth mentioning that several attempts have been made to develop more specific 'cerebroactive' agents[31] or new types of anti-ischaemic drugs[3,15], among them corticosteroids, modulators of arachidonate metabolism, gangliosides, monoamine modulators, opioid receptor antagonists, TRH and its analogs, NMDA receptor antagonists, platelet-activating factor antagonists, as well as antioxidants and free-radical scavengers[15]. The potential therapeutic impact of antioxidants and free-radical scavengers should be extended to CNS pathology in general. There is an increasing amount of experimental evidence that oxidative stress is a causal, or at least an ancillary, factor in the neuropathology of several adult neurodegenerative disorders, as well as in stroke, trauma, and seizures[11]. Understanding the relationship between oxidative stress and, for example, glutamate neurotransmission, could lead to the development of pharmacological interventions that disrupt this chain of pathological events without impairing excitatory neurotransmission[11,55]. It has also been shown that endogenous antioxidants, such as α-tocopherol (vitamin E), ubiquinols (coenzyme Q), retinoic acid (vitamin A) and ascorbic acid (vitamin C), are reduced following CNS trauma[43]. Coupled with the depletion of membrane-bound gangliosides, this loss of endogenous reductive capacity may permit an uncontrolled progression of enzymatic and peroxidative hydrolytic damage to cellular membranes. Reversal of this process provides the rationale for antioxidant replacement therapies[28,48,67].

Free radicals and programmed cell death (apoptosis)

The recent renewal of interest in free radicals comes from the evidence for a potential link between oxidative stress and programmed cell death (PCD) or apoptosis[5,11,60]. PCD and apoptosis are often used interchangeably to describe a mechanism of cellular death that is believed to play an important role in a wide variety of physiological situations, and that when dysregulated can contribute to the pathogenesis of many diseases, including AD. Solving the puzzle of apoptosis will not only improve our understanding of many basic biological processes, but might also have major therapeutic implications. A number of gene products have been implicated in the control of, or participation in, apoptosis[46]. Recently, the *bcl*-2 gene has emerged as a critical regulator of PCD in a variety of physiological and pathological contexts[19,61]. It appears that *bcl*-2 prevents cell death by decreasing the net cellular generation of reactive oxygen species[33,36,38]. An extended study of the relationship between *bcl*-2 control of PCD, generation of free radicals (with or without the participation of nitric oxide formation), and neurodegeneration seems to be a very promising approach for pharmacological modulation at this level. The therapeutic use of survival factors (that remain to be discovered) could well revolutionize the treatment of conditions in which cells die in the nervous system[60].

The nitric oxide connection: friend or foe?

Oxidative stress (possibly leading to neurodegenerative processes) can also generate nitric oxide (NO) via the calcium-dependent activation of NO-synthase, and this occurs when excess glutamate is released in an ischaemic situation[7]. The concomitant formation of superoxide/hydrogen peroxide might lead to a synergy between the cytotoxic effects of NO and these active oxygen species[71]. However, it has recently been shown that NO is able to protect against cellular damage and cytotoxicity from reactive oxygen species[81]. The question remains whether NO is 'friend or foe to the injured brain'[7]. Pharmacological modulation of NO-synthase activity or NO sequestration is now also feasible in CNS preparations, including cell cultures[20,32]. Thus, further investigation at the level of a possible synergistic or antagonist relationship between NO and reactive oxygen species may provide useful information about the neurodegenerative process and its treatment.

Animal models of AD and transgenic mice

Up to now, the neuropathological changes associated with AD have been studied using different experimental

animal models. These include rabbits, cats and rats treated with aluminium salts, inducing neurofibrillary degeneration; rats or monkeys injected with various neurotoxins (ibotenic, kainic, quinolic, quisqualic and N-methyl-D-aspartic acids); aged rats (sometimes aged monkeys) in which pathologies associated with normal aging may be detected[80]. In general, these animal models have been used to investigate the effectiveness or usefulness of specific pharmacotherapies. The drugs are often effective in the animal model but ineffective in patients with AD. Most attempts are directed only toward the improvement of the cholinergic deficit associated with AD[80] and tend to ignore the effects of degeneration in noncholinergic neural systems. These animal models may therefore be invalid because they do not mimic all of the pathological and neurochemical components associated with AD[80].

Consequently, the desire for a more appropriate animal model to study both the cause of AD and any potential treatments, has spurred several efforts over the past few years to engineer genetically mice that would mimic AD symptoms. These efforts did not meet with much success until last year. Recently, two groups have been able to introduce the βAPP human gene into mice that then make almost as much human as mouse βAPP[39,58]. Neither group has yet seen any sign that their mice are developing plaques or other types of AD pathology. It is quite possible that excess βAPP production may not be at fault in the first place. Both groups are now moving ahead to the next step, which is to insert into mice the mutant βAPP genes that have been linked to hereditary early-onset AD in some families. Whether these mutations can now cause pathology in mice is under intense investigation.

Note added in proof

During the final steps of the editorial process, I have become aware of the existence of a new type of acetylcholinesterase (ACHE) inhibitor, namely Ro 46–5934, which is also able to behave as a muscarinic M2-antagonist in vitro[4]. As a result, Ro 46–5934 would ameliorate cholinergic transmission by ACHE inhibition and blockade of a feed-back mechanism (through presynaptic M2 receptors) that reduces acetylcholine release.

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1 Ashall, F., and Goate, A.M., Role of the β-amyloid precursor protein in Alzheimer's disease. Trends biochem. Sci. *19* (1994) 42–46.
2 Barinaga, M., Neurotrophic factors enter the clinic. Science *264* (1994) 772–774.
3 Boddeke, E., Hugtenburg, J., Jap, W., Heynis, J., and Van Zwieten, P., New anti-ischaemic drugs: cytoprotective action with no primary haemodynamic effects. Trends pharmac. Sci. *10* (1989) 397–400.
4 Borroni, E., Damsma, G., Giovacchini, C., Mutel, V., Jakob-Rötne, R., and Da Prada, M., A novel acetylcholinesterase inhibitor, Ro 46–5934, which interacts with muscarinic M2 receptors. Biochem. Soc. Trans. *22* (1994) 755–758.
5 Buttke, T.M., and Sandstrom, P.A., Oxidative stress as a mediator of apoptosis. Immunol. Today *15* (1994) 7–10.
6 Chatellier, G., and Lacomblez, L., Tacrine (tetrahydroaminoacridine; THA) and lecithin in senile dementia of the Alzheimer type: a multicentre trial. Br. med. J. *300* (1990) 495–499.
7 Choi, D.W., Foe or friend to the injured brain? Proc. natl Acad. Sci. USA *90* (1993) 9741–9743.
8 Chun, M.R., and Mayeux, R., Alzheimer's disease. Curr Opin. Neurol. *7* (1994) 299–304.
9 Collerton, D., Cholinergic function and intellectual decline in Alzheimer disease. Neurosci. *19* (1986) 1–28.
10 Cook, P., and James, I., Cerebral vasodilators. New Engl. J. Med. *305* (1981) 1508–1513; 1560–1564.
11 Coyle, J.T., and Puttfarcken, P., Oxidative stress, glutamate, and neurodegenerative disorders. Science *262* (1993) 689–695.
12 Cummings, J.L., Clinical features and treatment of Alzheimer's disease. Curr.Opin. Neurol. Neurosurg. *3* (1990) 90–97.
13 Davis, K.L., Thal, L.J., Gamzu, E.R., Davis, C.S., Woolson, R.F., Gracon, S.I., et al., A double-blind, placebo-controlled multicenter study of tacrine for Alzheimer's disase. New Engl. J. Med. *327* (1992) 1253–1259.
14 Eagger, S.A., Levy, R., and Sahakian, B.J., Tacrine in Alzheimer's disease. Lancet *337* (1991) 989–992.
15 Faden, A.I., and Salzman, S., Pharmacological strategies in CNS trauma. Trends pharmac. Sci. *13* (1992) 29–35.
16 Farlow, M., Gracon, S.I., Hershey, L.A., Lewis, K.W., Sadowsky, C.H., and Dolan-Ureno, J., A controlled trial of tacrine in Alzheimer's disease. J. Am. med. Ass. *268* (1992) 2523–2529.
17 Franssen, E.H., Kluger, A., Torossian, C.L., and Reisberg, B., The neurologic syndrome of severe Alzheimer's disease. Relationship to functional decline. Archs Neurol. *50* (1993) 1029–1039.
18 Gandy, S., and Greengard, P., Amyloidogenesis in Alzheimer's disease: some possible therapeutic opportunities. Trends pharmac. Sci. *13* (1992) 108–113.
19 Garcia, I., Martinou, I., Tsujimoto, Y., and Martinou, J.C., Prevention of programmed cell death of sympathetic neurons by the bcl-2 proto-oncogene. Science *258* (1992) 302–304.
20 Garthwaite, J., Glutamate, nitric oxide and cell-cell signalling in the nervous system. Trends Neurosci. *14* (1991) 60–67.
21 Gauthier, S., Bouchard, R., Lamontagne, A., Bailey, P., Bergman, H., et al., Tetrahydroaminoacridine-lecithin combination treatment in patients with intermediate-stage Alzheimer's disease. New Engl. J. Med. *322* (1990) 1272–1276.
22 Gentleman, S.M., Graham, D.I., and Roberts, G.W., Molecular pathology of head trauma: altered βAPP metabolism and the aetiology of Alzheimer's disease. Prog. Brain Res. *96* (1993) 237–246.
23 Giacobini, E., Pharmacotherapy of Alzheimer's disease: New drugs and novel strategies, in: Alzheimer's Disease: Advances in Clinical and Basic Research, pp. 529–538. Eds B. Corain, K. Iqbal, M. Nicolini, B. Winblad, H. Wisniewski and P. Zatta. John Wiley & Sons Ltd. Chichester – New York – Brisbane – Toronto, Singapore 1993.
24 Goa, K.L., and Fitton, A., Velnacrine in Alzheimer's disease. CNS Drugs *1* (1994) 232–240.
25 Goate, A., Chartier-Harlin, M.C., Mullan, M., Brown, J., Crawford, F., Fidani, L. et al., Segregation of a missense mutation in the amyloid precursor protein gene with familial Alzheimer's disease. Nature *349* (1991) 704–706.
26 Goedert, M., Tau protein and the neurofibrillary pathology of Alzheimer's disease. Trends Neurosci. *16* (1993) 460–465.
27 Haass, C., and Selkoe, D.J., Cellular processing of β-amyloid precursor protein and the genesis of amyloid-β-peptide. Cell *75* (1993) 1039–1042.
28 Hall, E.D., Cerebral ischaemia, free radicals and antioxidant protection. Biochem. Soc. Trans. *21* (1993) 334–339.

29 Hammond, R.R., Gage, F.H., and Terry, R.D., Alzheimer's disease and spirochetes; a questionable relationship. NeuroReport *4* (1993) 840.

30 Haxby, J.V., Grady, C.L., Duara, R., Schlageter, N., Berg, G., and Rapoport, S.I., Neocortical metabolic abnormalities precede nonmemory cognitive defects in early Alzheimer's-type dementia. Archs Neurol. *43* (1986) 882–885.

31 Heise, G.A., Facilitation of memory and cognition by drugs. Trends pharmac. Sci. *8* (1987) 65–68.

32 Hirsch, D.B., Steiner, J.P., Dawson, T.M., Mammen, A., Hayek, E., and Snyder, S.H., Neurotransmitter release regulated by nitric oxide in PC-12 cells and brain synaptosomes. Curr. Biol. *3* (1993) 749–754.

33 Hockenbery, D.M., Oltvai, Z.N., Yin, X.-M., Milliman, C.L., and Korsmeyer, S.J., Bcl-2 functions in an antioxidant pathway to prevent apoptosis. Cell *75* (1993) 241–251.

34 Hoyer, S., Abnormalities in brain glucose utilization and its impact on cellular and molecular mechanisms in sporadic dementia of Alzheimer type, in: Alzheimers Disease: Amyloid Precursor Proteins, Signal Transduction, and Neuronal Transplantation, Ann. N.Y. Acad. Sci., vol. 695, pp. 77–80. Eds R.M. Nitsch, J.H. Growdon, S. Corkin, S. and R.J. Wurtman, New York Acad. Sciences, New York 1993.

35 Hung, A.Y., Haass, C., Nitsch, R.M., Qiu, W.Q., Citron, M., Wurtman, R.J., Growdon, J.H., and Selkoe, D.J., Activation of protein kinase C inhibits cellular production of the amyloid β-protein. J. biol. Chem. *268* (1993) 22959–22962.

36 Kane, D.J., Sarafian, T.A., Anton, R., Hahn, H., Gralla, E.B., Valentine, J.S., Örd, T., and Bredesen, D.E., Bcl-2 inhibition of neural death: decreased generation of reative oxygen species. Science *262* (1993) 1274–1277.

37 Katzman, R., and Saitoh, T., Advances in Alzheimer's disease. FASEB. J. *5* (1991) 278–286.

38 Korsmeyer, S.J., Shutter, J.R., Veis, D.J., Merry, D.E., and Oltvai, Z.N., Bcl-2/Bax: a rheostat that regulates an anti-oxidant pathway and cell death. Sem. in Cancer Biol. *4* (1993) 327–332.

39 Lamb, B.T., Sidodia, S.S., Lawler, A.M., Slunt, H.H., Kitt, C.A., Kearns, W.G. et al., Introduction and expression of the 400 kilobase precursor amyloid protein gene in transgenic mice. Nat. Genet. *5* (1993) 22–30

40 Lamy, P.P., The role of cholinesterase inhibitors in Alzheimer's disease. CNS Drugs *1* (1994) 146–165.

41 Lapchak, P.A., Nerve groth factor pharmacology: Application to the treatment of cholinergic neurodegeneration in Alzheimer's disease. Expl. Neurol. *124* (1993) 16–20.

42 Lee, V.M.-Y., Balin, B.J., Otvos L. Jr., and Trojanowski, J.Q., A68: a major subunit of paired helical filaments and derivatized forms of normal tau. Science *251* (1991) 675–678.

43 Lemke, M., Frei, B., Ames, B.N., and Faden, A.I., Decreases in tissue levels of ubiquinol-9 and 10, ascorbate and α-tocopherol following spinal cord impact trauma in rats. Neurosci. Lett. *108* (1990) 201–206.

44 Lowe, G.D.O., Drugs in cerebral and peripheral arterial disease. Lancet *300* (1990) 524–528.

45 Mandelkow, E.-M., and Mandelkow, E., Tau as a marker for Alzheimer's disease. Trends biochem. Sci. *18* (1993) 480–483.

46 Martin, S.J., Green, D.R., and Cotter, T.G., Dicing with death: dissecting the components of the apoptosis machinery. Trends biochem. Sci. *19* (1994) 26–30.

47 Marx, J., Alzheimer's debate boils over. Science *257* (1992) 1336–1338.

48 Mattson, M.P., Cheng, B., and Smith-Swintosky, V.L., Mechanisms of neurotrophic factor protection against calcium- and free radical-mediated excitotoxic injury: implications for treating neurodegenerative disorders. Expl Neurol. *124* (1993) 89–95.

49 Miklossy, J., Alzheimer's disease - a spirochetosis? NeuroReport *4* (1993) 841–848.

50 Miklossy, J., Kasas, S., Janzer, R.C., Ardizzoni, F., and Van der Loos, H., Further ultrastructural evidence that spirochaetes may play a role in the aetiology of Alzheimer's disease. NeuroReport *5* (1994) 1201–1204.

51 Molloy, D.W., and Cape, R.D.T., Acute effects of oral pyridostigmine on memory and cognitive function in SDAT. Neurobiol. Aging *10* (1989) 199–204.

52 Mullan, M., and Crawford, F., Genetic and molecular advances in Alzheimer's disease. Trends Neurosci. *16* (1993) 398–402.

53 Namba, Y., Tomonaga, M., Kawasaki, H., Otomo, E., and Ikeda, K., Apolipoprotein E immunoreactivity in cerebral amyloid deposits and neurofibrillary tangles in Alzheimer's disease and kuru plaque amyloid in Creutzfeldt-Jakob disease. Brain Res. *541* (1991) 163–166.

54 Nieto, A., Montejo de Garcini, E., Correas, I. and Avila, J., Characterization of tau protein present in microtubules and paired helical filaments of Alzheimer's disease patients's brain. Neuroscience *37* (1990) 163–170.

55 Olanow, C.W., A radical hypothesis for neurodegeneration. Trends Neurosci. *16* (1993) 439–444.

56 Olson, L., NGF and the treatment of Alzheimer's disease. Expl Neurol. *124* (1993) 5–15.

57 Palmer, A.M., and DeKosky, S.T., Monoamine neurons in aging and Alzheimer's disease. J. neural Transm. *91* (1993) 135–159.

58 Pearson, B.E., and Choi, T.K., Expression of the human β-amyloid precursor protein gene from a yeast artificial chromosome in transgenic mice. Proc. natl Acad. Sci. USA *90* (1993) 10578–10582.

59 Pollard, H.B., Rojas, E., and Arispe, N., β-Amyloid in Alzheimer's disease. CNS Drugs *2* (1994) 1–6.

60 Raff, M.C., Barres, B.A., Burne, J.F., Coles, H.S., Ishizaki, Y., and Jacobson, M.D., Programmed cell death and the control of cell survival: Lessons from the nervous system. Science *262* (1993) 695–700.

61 Reed, J.C., Bcl-2 and the regulation of programmed cell death. J. Cell Biol. *124* (1994) 1–6.

62 Roberts, F., and Lazareno, S., Cholinergic treatments for Alzheimer's disease. Biochem. Soc. Trans. *17* (1989) 76–79.

63 Rogers, J., Kirby, L.C., Hempelman, S.R., Berry, D.L., McGeer, P.L., Kaszniak, A.W., Zalinski, J., Cofield, M., Mansukhani, L., Willson, P., and Kogan, F., Clinical trial of indomethacin in Alzheimer's disease. Neurology *43* (1993) 1609–1611.

64 Rogers, J., Inflammation and Alzheimer's disease. CNS Drugs *1* (1994) 241–244.

65 Royston, M.C., Rothwell, N.J., and Roberts, G.W., Alzheimer's disease: pathology to potential treatments? Trends pharmac. Sci. *13* (1992) 131–133.

66 Schmechel, D.E., Saunders, A.M., Strittmatter, W.J., Crain, B.J., Hulette, C.M., Joo, S.H., Pericak-Vance, M.A., Goldgaber, D., and Roses, A.D., Increased amyloid β-peptide deposition in cerebral cortex as a consequence of apolipoprotein E genotype in late-onset Alzheimer disease. Proc. natl Acad. Sci. USA *90* (1993) 9649–9653.

67 Schubert, D., Kimura, H., and Maher, P., Growth factors and vitamin E modify neuronal glutamate toxicity. Proc. natl Acad. Sci. USA *89* (1992) 8264–8267.

68 Selkoe, D.J., Biochemistry of altered brain proteins in Alzheimer's disease. A. Rev. Neurosci. *12* (1989) 463–490.

69 Selkoe, D.J., Physiological production of the β-amyloid protein and the mechanism of Alzheimer's disease. Trends Neurosci. *16* (1993) 403–409.

70 Shutske, G.M., Pierrat, F.A., Cornfeldt, M.L., Szewczak, M.R., Huger, F.P., Bores, G.M., Harontanian, V., and Davis, K.L., (±)-9-Amino-1,2,3,4-tetrahydroacridin-1-ol. A potential Alzheimer's disease therapeutic of low toxicity. J. med. Chem. *31* (1988) 1278–1279.

71 Sies, H., Strategies of antioxidant defense. Eur. J. Biochem. *215* (1993) 213–219.

72 Slotkin, T.A., Nemeroff, C.B., Bissette, G., and Seidler, F.J., Overexpression of the high affinity choline transporter in cortical regions affected by Alzheimer's disease. Evidence from rapid autopsy studies. J. clin. Invest. *94* (1994) 696–702.

73 Spagnoli, A., and Tognoni, G., "Cerebroactive" drugs. Clinical pharmacology and therapeutic role in cerebrovascular disorders. Drugs *26* (1983) 44–69.

74 Steiner, B., Mandelkow, E.M., Biernat, J., Gustke, N., Meyer, H.E., Schmidt, B., Mieskes, G., Söling, H.D., Drechsel, D., Kirschner, M.W., Goedert, M., and Mandelkow, E., Phosphorylation of microtubules associated protein tau: identification of the site for Ca^+ -calmodulin dependent kinase and relationship with tau phosphorylation in Alzheimer tangles. EMBO J. *9* (1990) 3539–3544.

75 Summers, W.K., Majovski, L.V., Marsh, G.M., Tachiki, K., and Kling, A., Oral tetrahydroaminoacridine in long-term treatment of senile dementia. New Engl. J. Med. *315* (1986) 1241–1245.

76 Uterman, G., The apolipoprotein E connection. Curr. Biol. *4* (1994) 362–365.

77 Vincent, I.J., and Davies, P., A protein kinase associated with paired helical filaments in Alzheimer's disease. Proc. natl Acad. Sci USA *89* (1992) 2878–2882.

78 Walsh, T.J., Site-specific pharmacology for the treatment of Alzheimer's disease. Expl Neurol. *124* (1993) 43–46.

79 Watkins, P.B., Zimmerman, H.J., Knapp, M.J., Gracon, S.I., and Lewis, K.W., Hepatotoxic effects of tacrine administration in patients with Alzheimer's disease. J. Am. med. Assoc. *271* (1994) 992–998.

80 Wenk, G.L., Animal models of Alzheimer's disease, in: Animal Models of Neurological Disease, I Neurodegenerative Diseases, vol. 21, pp. 29–63. Eds A.A. Boulton, G.B. Baker, and R.F. Butterworth, Neuromethods, Humana Press, Totowa, New Jersey 1992.

81 Wink, D.A., Hanbauer, I., Krishna, M.C., De Graff, W., Gamson, J., and Mitchell, J.B., Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. Proc. natl Acad. Sci. USA *90* (1993) 9813–9817.

82 Yoshida, S., and Suzuki, N., Antiamnesic and cholinomimetic side-effects of the cholinesterase inhibitors, physostigmine, tacrine and NIK-247 in rats. Eur. J. Pharmac. *250* (1993) 117–124.