OVERVIEW AND PERSPECTIVE ON THE THERAPY OF ALZHEIMER’S DISEASE FROM A PRECLINICAL VIEWPOINT

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Contents

Abstract
1. Introduction: The Features of Alzheimer’s Disease
2. Animal Models
2.1 Lesions of the Forebrain Cholinergic System
2.2 Intracerebral β-Amyloid Injections
2.3 Transgenic Mice Overexpressing β-Amyloid And Presenilin1
2.4 Induction of a Brain Inflammation
2.5 Aging Animals
2.6 Glucose and Energy Metabolism Impairment, Phosphatase Inhibition
3. Conclusions
References

Abstract


1. Drugs effective in Alzheimer’s disease (AD) should have several aims: to improve the cognitive impairment, control the behavioural and neurological symptoms, delay the progression of the disease, and prevent the onset. In order to attain these targets, cell and animal models are needed on which to test pathogenetic hypothesis and demonstrate the potential effectiveness of new drugs. This overview examines the results obtained in animal models. They are the link between the molecular and biochemical studies on the disease and the reality of human pathology.
2. The development of animal models reproducing the complexity of AD pathogenetic mechanisms and clinical symptoms still represents a challenge for the preclinical investigators. Moreover, the succession of different animal models well documents the progressive widening of our knowledge of the disease with the identification of new therapeutic targets.

3. The main animal models are listed, and their contribution to the understanding of the pathogenic mechanisms and development of the drugs presently used in AD therapy is described. Moreover, their role in the study of future drugs is analysed.

4. Preclinical studies on cholinesterases and animal models mimicking the cholinergic hypofunction occurring in AD have been instrumental in developing cholinesterase inhibitors, which are the only recognised drugs for the symptomatic treatment of AD.

5. Artificially created β-amyloid (Aβ) deposits in normal rats, and transgenic mice overexpressing amyloid precursor protein (APP) are the models on which the future treatment are tested. They are aimed to prevent formation of Aβ deposits or its transformation in neuritic plaques.

6. Models of brain inflammation, aging animals, and models of brain glucose and energy metabolism impairment make it possible to identify and assess the activity of anti-inflammatory agents, antioxidants, ampakines and other potentially active agents.

7. It is concluded that the present level of information on AD could never have been reached without preclinical studies, and the development of new drugs will always require extensive preclinical investigations.

Keywords: Alzheimer’s disease, animal models, β-amyloid, cholinesterase inhibitors

Abbreviations: acetylcholine (ACh), Alzheimer’s disease (AD), amyloid precursor protein (APP), Apolipoprotein E (ApoE), β-amyloid (Aβ), choline acetyltransferase (ChAT), cholinesterase inhibitor (ChEI), gamma-aminobutyric acid (GABA), nonsteroidal anti-inflammatory drugs (NSAIDs), nucleus basalis of Maynert (NB).

1. Introduction: The Features of Alzheimer’s Disease

Preclinical studies in Alzheimer’s Disease (AD) have two purposes: to investigate its pathogenetic mechanisms, and identify potentially effective drugs, their mechanisms of action and toxicity. The final aim is to alleviate the individual suffering caused, and reduce the social burden represented by this disease, which may last for many years. According to the DSM-IV (1994), it is estimated that in U.S. 2% - 4% of the population over age 65 years have Dementia of Alzheimer’s Type. The prevalence seems even higher in some Western countries. The Italian Longitudinal Study on Aging (ILSA) (1997) reports a prevalence of 6.4% in Italy in the population over age 65.

The preclinical investigations on AD must consider the complexity of the symptoms of the disease and of their neuropathological substrates whose causes and pathogenesis are still partly unknown.

The “Diagnostic criteria for dementia of Alzheimer’s type” of the DSM-IV defines the disease in the first place by its multiple, progressive, cognitive deficits. However, behavioural disturbances are frequently present, and subtypes are identified in which delirium, delusions and depressed mood can be superimposed on the dementia. It must be reminded that in the first patient described in 1907 by Alois Alzheimer, one of the first disease symptoms was "a strong feeling of jealousy towards her husband......[and that] sometimes [she] thought that people were out to kill her...." (Bick et al. 1987).
Therefore a symptomatic therapy must aim to three targets, the cognitive impairment, the behavioural disturbances and the neurological symptoms, such as sleep alterations and deglutition impairment which may be also present.

The cognitive, psychiatric, and neurological symptoms, which make AD such a difficult disease to treat, are the consequence of profound, widespread histological and anatomical alterations, associated with typical neurochemical changes. The two main histopathological hallmarks of AD are the β-amyloid (Aβ) plaques and the neurofibrillary pathology. The exact relationship between these pathological features is still obscure, although it is clear that β-amyloid plaques precede neurofibrillary tangles in neocortical areas (Vickers et al., 2000). The amyloid cascade hypothesis implies that deposition of Aβ-protein is the causative agent of AD pathology which is followed by neurofibrillary pathology, cell loss and dementia (Hardy and Higgins, 1992). The Aβ plaque formation evokes a diffuse brain inflammatory response (McGeer and McGeer, 1995). The inflammation mediators, including cytokines, components of the complement cascade and acute phase reactants, released in the brain may set in motion a self-stimulating cycle leading to further neuronal death, amyloid deposition and subsequent plaque formation (Griffin et al., 1998).

Irrespective of the pathogenetic mechanism, the key pathological change in the brain, linked to the development of dementia, is the gradual degeneration of nerve cells and the related loss of specific synaptic connections (Vickers et al. 2000). This process is highly selective for certain brain regions and types of nerve cells. The forebrain cholinergic neurones were the first group of cells whose degeneration has been demonstrated in AD brain (Davies and Maloney, 1976; Arendt et al., 1983). The involvement of other subcortical pathways involving catecholaminergic and serotonergic transmission, of a subset of pyramidal cells using glutamate as neurotransmitters and of specific areas such as the amygdala, the hippocampus and others has been since then demonstrated (see ref. in Vickers et al., 2000).

Clinical and neuropathological observations have been and still are the initial and principal source of information for the description and understanding of AD. However, preclinical investigations have offered the possibility to interpret the clinical findings and to build working hypothesis on which to develop effective drugs.

Drugs effective in AD should have several aims:
1 Improve the cognitive impairment
2 Control the behavioural and neurological symptoms
3 Delay the progression of the disease
4 Prevent the onset

In order to attain these targets, cell and animal models are needed on which to test pathogenetic hypothesis and demonstrate the potential effectiveness of new drugs. In this overview, emphasis will be given to the results obtained in animal models which are the link between the molecular and biochemical studies on the disease and the reality of human pathology.

### 2. Animal Models

The development of animal models reproducing the complexity of the pathogenetic mechanisms and clinical symptoms of AD represented and still represent a challenge for the preclinical investigators. Moreover, the succession of different animal models well documents the progressive widening of our knowledge of the disease with the identification of new therapeutic targets. The advantages and limitations of the different models have been matter of several reviews and proceedings (Hoyer et al., 1994a; Emilien et al., 2000).

The most important animal models for AD research are listed in Table 1. In the following paragraphs, the contribution of the different models to the understanding of the disease and its treatment and their importance for future developments will be discussed.
Lesions of the forebrain cholinergic system

Intracerebral β-amyloid injections

Transgenic mice overexpressing β-amyloid and presenilin1

Induction of a brain inflammation

Aging animals

Glucose and energy metabolism impairment, phosphatase inhibition

2.1 Lesions of the Forebrain Cholinergic System

The discovery that in AD there is a characteristic hypofunction of the forebrain cholinergic system was made by clinicians and neuropathologists (David and Maloney, 1976). It was a seed which fell on a ground broken up by years of preclinical work and its fruit was the "cholinergic hypothesis of geriatric memory dysfunction" (Bartus et al., 1982). The cholinergic hypothesis was the theoretical basis for the cholinergic therapy, which almost fifteen years later resulted in the cholinesterase inhibitors tacrine, donepezil, rivastigmine the first drugs approved by the Food and Drug Administration specifically for AD (Giacobini 2000).

Toward the end of the 70's, the extraction and purification of choline acetyltransferase (ChAT) made it possible to produce specific antibodies by which to visualise the cholinergic neurons and map the cholinergic nuclei and pathways. The forebrain nuclei, including the nucleus of Meynert, were identified as the main source of the cortical and hippocampal cholinergic innervation (Lehman et al., 1980). Their degeneration is the cause of the cholinergic deficits in AD.

The hypothesis that a cholinergic deficits might be responsible for memory impairment was based on studies showing learning and memory deficits in animals and humans whose cholinergic system was blocked by atropine or scopolamine (Pazzagli and Pepeu, 1964; Drachman 1977). Confirmation was sought by animal investigations in which the forebrain cholinergic nuclei were destroyed, initially by electrolytic lesions (Lo Conte et al., 1982), then by excitotoxins (Casamenti et al., 1988) and finally by the selective immunotoxins 192 IgG-saporin (Wenk et al., 1994). Intracerebroventricular injection of 192 IgG-saporin brings about a widespread degeneration of cholinergic neurons including the nucleus basalis of Meynert (NB) and the septum (Lin et al., 1999). The reversion of the learning and memory deficits induced by NB lesions has become a classical preclinical test for the screening of drugs aimed to correct the cholinergic hypofunction in AD (see ref. in Pepeu, 2000).

Animals with lesions of the forebrain cholinergic nuclei represent a useful model of cholinergic hypofunction. However, as a model of AD, it shows many limitations. Namely, the cholinergic hypofunction is not associated with the formation of Aβ plaques and neurofibrillary tangles, and there is no progressive worsening of the hypofunction and cognitive deficit, but some recovery takes place (Casamenti et al. 1988). However, it has been shown that the cholinergic hypofunction is associated with an increase in the β-amyloid precursor protein (APP) whose levels in the cortex can be directly correlated with the escape latency in a water maze (Lin et al., 1999) The APP increase can be prevented by the administration of a muscarinic agonist for 8 days. Beach et al. (2000) demonstrated that cholinergic deafferentation of the rabbit cortex leads to Aβ deposition in the cerebral blood vessels and perivascular neuropil. These preclinical studies demonstrate in vivo that the cholinergic hypofunction, even if it is not the primary cause of Aβ deposition, may enhance it. They also support the hypothesis that the cholinergic therapy may not only improve the cognitive deficits but also delay the progression of the disease, as proposed by Nitsch et al.(1992) on the basis of experiments on cell culture.
The role of the forebrain cholinergic system in learning and memory, particularly in information storage and recall is not yet fully unravelled, in spite of the extensive number of investigations (Everitt and Robbins, 1997). Therefore, preclinical studies on animals with cholinergic hypofunction induced by selective lesions of the NB and/or septal nuclei are useful tools for defining which types and components of memory depend on the forebrain cholinergic pathways, which neurotransmitter systems are modulated by the cholinergic system, and which drugs can effectively restore the cholinergic hypofunction and behavioral impairment.

2.2 Intracerebral β-amyloid injections

The Aβ cascade hypothesis proposed by Hardy and Higgins (1992) as the pathogenetic hub of AD is based on the assumption that Aβ is neurotoxic. A large number of in vitro and in vivo preclinical investigations have been devoted to demonstrating the toxicity of the Aβ peptides and its neurotoxic mechanism. Aβ is a proteolytic fragment of β-APP constituted by 39-43 amino acids which forms amyloid fibrils (Selkoe, 1993). The most conventional approaches for demonstrating the neurotoxicity of Aβ peptides are to add them to primary neuronal cultures, to inject them into specific brain areas or infuse them through the cerebral ventricles. It is beyond the scope of this paper to review the extensive literature which has resulted from these experimental approaches and has been the object of a recent overview (Harkany et al., 1999a).

Abe et al. (1994) demonstrated that the injection of synthetic Aβ peptides β(12-28), β(25-35) and β(1-40) into the septum of adult rats induced a marked decrease in basal and K+-evoked acetylcholine (ACh) release in the hippocampus. Similar injections of β(25-35) and β(1-40) peptides into the nucleus basalis resulted in a Congo Red positive deposit associated with a decrease in the number of ChAT-positive neurons in the surrounding area, a decrease in ACh extracellular levels in the cortex, and a delayed disruption of object recognition, indicating a cognitive impairment (Giovannelli et al., 1995). The peptide deposit was aggregated in a fibrillary form, as confirmed by electron microscopy analysis, four months after injection, whereas at six months the fibril organisation was lost. Concomitant with the loss of fibril conformation, a complete recovery in the number of ChAT positive neurons in the nucleus basalis and ACh release in the cortex was observed (Giovannelli, 1998). A delayed impairment of an operant behavior was also observed from 50 days after the injection of aggregated Aβ(1-42) (O'Hare et al., 1999).

These findings confirmed in vivo the neurotoxic effects of Aβ observed in primary neuronal cell cultures exposed to Aβ-peptides (Koh et al., 1990; Yanker et al., 1990). The multiple mechanisms through which Aβ-peptides, involving oxidative stress, loss of cellular calcium homeostasis and mitocondrial dysfunction, have been reviewed by Mattson and Pedersen (1998). Indirect demonstration of the importance of the oxidative stress in Aβ toxicity was given by the protective effects of the antioxidants α-tocopherol and idebenone on the learning and memory deficits induced by Aβ(1-42) intraventricular chronic infusion (Yamada et al. 1999). In most studies only the toxicity for the cholinergic system was investigated. However, a delayed decrease in glutamic acid decarboxylase activity was detected in the NB injected with Aβ(1-40) and was associated with an increase in basal and K+-evoked cortical gamma-aminobutyric acid (GABA) release (Scali et al., 1999), a finding demonstrating that different neurons are vulnerable to Aβ.

In both in vitro and in vivo experiments it has been shown that Aβ neurotoxicity depends on its fibrillar aggregation forming β-sheet (Busciglio et al., 1992; Giovannelli et al., 1998). It is assumed that by precluding the fibrillar aggregation and β-sheet conformation it might be possible to prevent Aβ deposition and plaque formation. The different approaches have been reviewed by Harkany et al. (1999b). Recently, this author demonstrated that a tetrapeptide Aβ antagonist, infused by reverse microdialysis into the NB of adult rats, attenuated the excitotoxic action of Aβ(1-42). Soto et al. (2000) demonstrated that intracerebral co-injections of β-sheet breakers peptides and Aβ (1-42) prevented the formation of neurotoxic deposits.

In conclusion, these preclinical experiments give support to the hypothesis of the pivotal pathogenetic role of Aβ deposit in AD, throw some light on the molecular mechanisms of Aβ toxicity, and offer an
experimental model for testing potentially useful drugs. Undoubtedly, there are limitations in the validity of intracerebral Aβ injections as an AD model. The main criticism is that the concentrations of Aβ-peptides created by adding them to cell cultures or injecting them into the brain are much higher than those existing in the brain and CSF of AD patients (Vickers et al., 2000). Nevertheless, with this caveat, animals in which Aβ deposits have been artificially made remain a useful preclinical tool.

2.3 Transgenic Mice Overexpressing β-amyloid and Presenilin 1

The development of transgenic mice, mimicking the genetic mutations occurring in familial AD and showing some of the neurochemical and morphological alterations of AD, is another example of the strict interactions between clinical and preclinical investigations into this disease. The clinical and genetic investigations have identified the early onset, familial forms of AD, and the genes in which autosomal dominant mutations take place. Mutations of genes on chromosomes 1, 14 (presenilin 1 and 2), and 21 (APP) cause the familial forms of AD. An allelic polymorphism on chromosome 19 (Apolipoprotein E) affects the age of onset of sporadic AD. The preclinical investigations contributed with the biotechnological know-how for generating transgenic animals. The result is an animal model providing strong support to the view that Aβ formation is an early and critical pathogenic event in AD progress (Emilien et al., 2000).

Two recent reviews exhaustively describe the theoretical background, techniques for generating the transgenic mice, and their characteristics (Seabrook and Rosahl, 1999; van Leuven, 2000). An example of the importance of the transgenic mice for understanding AD pathogenesis is represented by a recent paper by Bales et al. (1999). In this work, the role of ApoE in Aβ deposition has been demonstrated in transgenic mice overexpressing the v717 human amyloid precursor protein (APP), crossed with ApoE knockout mice. No Aβ deposits were found in any brain region of mice with no ApoE alleles, at various ages, and a relationship existed between the extension of Aβ deposits and the number of ApoE alleles. Moreover, by comparing transgenic mice in which a human APP mutation was associated with a familial AD (FAD) mutation, with wild-type humanAPP mice, it was demonstrated (Mucke et al., 2000) that Aβ is synaptotoxic even in the absence of plaques, since both mouse strains showed increased Aβ levels and a reduced synaptic density, but only the FAD-mutant humanAPP mice formed Aβ plaques.

Even transgenic mice have limitations as AD models. For instance, none of the transgenic animals overexpressing the AD-causing mutation of APP has shown neurofibrillary tangles formation (Marsel-Mesulam, 1999). Moreover, of the 5 transgenic mouse models overexpressing Aβ, listed by Emilien (2000) in his review, only one showed behavioural deficits (Hsiao et al., 1996). Moreover, no loss of cholinergic neurons has been observed so far in the transgenic mice. Only dystrophic cholinergic fibres in the vicinity of neuritic plaques have been observed by Sturchler-Pierrat et al. (1997) in APP mutant mice, and a reorganisation of the cholinergic terminals has been described in the cerebral cortex of doubly transgenic mice overexpressing APP and presenilin1 genes (Wong, 1999). In both types of transgenic mice, severity of the cholinergic alterations is far from that found in AD. These findings leave open the question whether, and under which conditions Aβ is toxic for the cholinergic neurons.

If transgenic mice overexpressing APP and showing high Aβ levels help in understanding the pathogenic role of Aβ, APP-null mice are a useful tool for investigating the physiological role of APP. APP-null mice show age-related cognitive deficits. To which extent the deficits depends on synaptic boutons loss is matter of debate (Dawson et al., 1999; Phinney et al., 1999). Nevertheless, these results support the hypothesis that APP exert a trophic role in the brain.

Transgenic mice represent a useful model for testing drugs active in preventing Aβ deposition and plaque formation. However, presumably due to the difficulty to obtain the large number of animals needed for drug testing, transgenic mice have been used so far only for testing the efficacy of immunization with Aβ against Alzheimer-disease-like pathology. Immunisation with synthetic human Aβ(1-42) of young mice over-expressing a mutant human APP prevented almost completely Aβ deposition. (Schenk et al., 1999). This is an example of the importance of preclinical studies for creating the experimental premises needed for testing a new treatment in man.
2.4 Induction of a Brain Inflammatory Response

Brain inflammation is considered a pathogenetic link in many neurodegenerative diseases, including AD (McGeer and McGeer, 1995; Kreutzberg, 1996). Neuropathological studies revealed that the neuritic plaques are associated with reactive microglia and astrocytes as well as dystrophic neurites (McGeer and Rogers, 1992; Mackenzie and Munoz, 1998). Epidemiological and clinical studies (ref. in McGeer and McGeer, 1998), reporting the efficacy of nonsteroidal anti-inflammatory drugs (NSAIDs) in reducing the incidence and progression of AD, provided strong support for the critical involvement of inflammatory processes in the pathogenesis of AD. It has been proposed that Aβ deposits in the neuritic plaques trigger an inflammatory response of the adjacent glial population, which in turn, sets into motion a cascade of glial-mediated neuropathogenetic factors (Stanley et al., 1994).

The neuropathological post-mortem observations of the brain inflammatory response in AD, together with the epidemiological studies demonstrating a reduced risk of developing AD in patients taking NSAIDs for peripheral rheumatic diseases, arose hopes of therapeutic interventions and prompted a large number of preclinical studies aimed to investigate whether:

1) Aβ could activate microglia and bring about an inflammatory response,

2) the inflammatory response is neurotoxic,

3) NSAIDs could control brain inflammatory response.

Giovannelli et al. (1995) demonstrated that the injection of the Aβ(25-35) and (1-40) peptides into the nucleus basalis of adult rats was followed by a strong, long lasting, astroglial reaction associated with a decrease in the number of cholinergic neurons. Microglia activation by Aβ (1-40), under the same experimental conditions, was described by Scali et al. (1999). Substantial astroglial reaction was observed by O’Hare et al., (1999) after intrahippocampal injection of aggregated Aβ (1-42). Astrocytosis and microgliosis has been described in the brain of transgenic mice showing abundant Aβ deposits (Emilien et al., 2000; Schenk et al., 1999). Taken together these results confirm that Aβ deposits induce a brain inflammatory response.

The second question is whether the inflammatory response is neurotoxic.

According to Hauss- Wegrzyniak et al. (1998) a long-term lipopolysaccharide (LPS) infusion into the 4th ventricle was followed by astrocyte activation, an increase in microglia cells, an increase in the levels of interleukin-1β, tumor necrosis factor-α, APP mRNA and the degeneration of hippocampal neurons. Electron microscopic studies of neurons within the hippocampus revealed ultrastructural changes that suggested impaired or reduced synthesis of cellular proteins within the cytoplasm (Hauss-Wegrzyniak et al.,2000). It was also demonstrated that chronic neuroinflammation induced by LPS produced a time-dependent but not dose-dependent degeneration of the nucleus basalis cholinergic cells (Willard et al.,1999).

The inflammatory response induced by the injection of interleukin-1β in the nucleus basalis did not reduce cortical ACh release, measured by microdialysis 30 days post-injection, but increased GABA release (Casamenti et al. 1999). Perlmutter et al. (1991) found no changes in either ChAT activity in the cortex or in the number of basal forebrain ChAT immunopositive cells, after infusion of LPS into the forth ventricle for four weeks. From these results it may be assumed that a brain inflammatory response is not immediately neurotoxic. However, if the inflammation persists with the associated release of cytokines, glutamate and NO, a neuronal degeneration may slowly develop.

The last question is whether NSAIDs control the brain inflammatory response. According to Scali et al. (2000), seven days of treatment with the NSAID nimesulide strongly attenuated the microglial reaction, reduced the number of inducible nitric oxide synthase-positive cells and completely abolished the increase in prostaglandin-E2 formation in rats receiving a unilateral injection of the excitotoxin quisqualic acid in the nucleus basalis. Administration of nitrofuribiprofen for 30 days dose-dependently, and significantly, attenuated in rats the brain inflammation induced by intracerebroventricular injection of LPS, as indicated by a decreased density and reactive state of microglial cells in rats. Nitroaspirine was less effective (Hauss-Wegrzyniak et al.,1999).
In conclusion, the preclinical experiments demonstrate that brain inflammation is a striking, complex neurobiological event involving different cells and a large number of active substances. NSAIDs are able to attenuate brain inflammatory response, as effectively as in peripheral tissues. However, so far the therapeutic trials with anti-inflammatory agents in patients affected by AD have not been successful (Scharf et al., 1999; Aisen et al, 2000) at variance with the epidemiological demonstrations of the efficacy in reducing the incidence and progression of AD (ref. in McGeer and McGeer, 1998). Since inflammation appears to require a long time in order to induce neuronal damage, it is possible that the contradiction between preclinical positive results and favourable epidemiological studies on one side, and negative therapeutic results in AD patients might depend on the different time scales of the events investigated.

2.5 Aging Animals

Aging animals have been and are the most common for investigating drugs potentially active on AD. Old mice and rats are used most frequently since they are easy to obtain and relatively cheap. Old monkeys have been also used, and remain the last preclinical step before clinical trials. Usefulness, constraints and limits of studies on aging animals have been matter of discussions in the early days of research on aging and dementia (Finch, 1982, Pepeu et al., 1986). Much work has been devoted to investigating the aging of the brain cholinergic system (Pepeu et al., 1993) as a model of the cholinergic hypofunction occurring in AD.

There are two main limitations of the aging animal model. First, aging animals do not develop the neuropathological picture of AD. Rare plaques have been described in the monkey, but none of the histopathological alterations typical of AD occur in aging rodents. Second, in aging animals, it is relatively easy to obtain an improvement of the cholinergic hypofunction and the cognitive deficits with drugs whose efficacy is difficult to demonstrate in clinical trials.

This is the case of phosphatidylserine, which administered to aging rats restores brilliantly the cholinergic hypofunction and the cognitive deficits. Unfortunately, the clinical trials have been much less successful, even if some therapeutic activity has been demonstrated (see ref. in Pepeu et. al 1996). Intracerebral NGF administration restores the cholinergic neuron atrophy and memory impairment in aging rats (Fisher et al.,1987) but the improvement in man is limited and its use requires intracerebroventricular injections (Olson et al., 1992). Similar is the situation with GM1 ganglioside (Svennerholm, 1994). Nevertheless, the observation that in rodents the age-associated loss of cholinergic neurons can be corrected is important not only for the therapy of AD but for possible interventions on age associated memory impairment. Moreover, this is an interesting neurobiological issue since it indicates that the cholinergic neurons in the aging rats do not disappear but stop expressing ChAT. Does something similar also happen in the aging human brain?

Among the drugs which are effective in restoring the cognitive deficits in aging rats there are the ampakines (Granger et al., 1996; Bartolini et al., 1996), drugs which prolong the action of glutamate on its AMPA receptors (Ito et al., 1990). Among them aniracetam, piracetam and others of the so called “nootropic” agents. Their therapeutic value still needs to be clearly demonstrated.

Finally, a validation of the aging animals as a tool for testing new drugs potentially active on AD comes from the observation that cholinesterase inhibitors (ChEI) correct both cholinergic hypofunction and cognitive deficits in aging animals (see ref. in Pepeu, 2000). For this reason demonstration of activity in aging animals is an unavoidable step in the development of new drugs for AD.

2.6 Glucose and Energy Metabolism Impairment

The hypothesis that AD might be the expression of metabolic alterations has been repeatedly proposed (Finch and Cohen, 1997). Emphasis has been placed on the changes in the energy/glucose metabolism (Hoyer et al., 1994b) and it has been suggested that type 2 diabetes may be a risk factor for AD development (Ott et al., 1996). A severe decrease in brain glucose utilisation (Hoyer et al., 1991) and a decrease in the function of the pyruvate dehydrogenase (Sorbi et al., 1983) has been demonstrated in AD brain. On the basis of these observations, glucose metabolism has been disrupted in rat brain by intracerebroventricular administration of streptozotocin with the aim to reproduce AD metabolic
Overview and perspective on the therapy of Alzheimer's disease

alterations. A comparison between the metabolic changes in glucose/energy metabolism found in sporadic AD and in rat brain 3 weeks after streptozotocin administration revealed remarkable analogies (Hoyer et al., 1994b), also including cognitive impairment (Lannert et al., 1998). Intracerebral injections of bromopyruvate have been used in order to inhibit pyruvate dehydrogenase. The inhibition of the tricarboxylic acid cycle was accompanied by cognitive impairment (Hoyer et al., 1986). None of the typical neuropathological features of AD developed in the brain of rats with an impairment of glucose metabolism but no long term studies have been carried out. The predictive value of the streptozotocin model in drug testing has been assessed only with estradiol (-17β) which, injected daily for about two weeks, attenuates both metabolic and behavioral impairments (Lannert et al., 1998).

The general impression derived from these preclinical experiments is that a derangement of glucose and energy metabolism is not at the heart of AD pathogenesis. However, it may be an important co-factor in aging and AD. Animals with an impaired glucose metabolism may be a useful model for demonstrating the activity of drugs such as Ginkgo biloba alkaloids whose actions are not yet well defined (Hoyer et al., 1999) or radical scavengers.

Although deposits of Aβ have been induced either by direct injection of the peptides or by creating transgenic mice, the formation of both molecular hallmarks of AD, abnormally high phosphorylated tau protein, forming the fibrillary tangles, and Aβ, have not been mimicked together in an experimental animal. In AD, activity of the protein phosphatase 2A, which under in vitro conditions is able to dephosphorylate paired helical filament-tau, appears to be reduced in both grey and white matter (Gong et al., 1993). A short but detailed analysis of the phosphorylation-dephosphorylation processes in both tau and Aβ generation was made by Arendt et al. (1995) who attempted to inhibit chronically phosphatase 1 and 2 by infusing okadaic acid in the rat lateral ventricle for two weeks. Okadaic acid is a specific inhibitor of the serine/threonine protein phosphatase 1 and 2A (Bialojan and Takai, 1988). According to Arendt et al., (1995,1998) the infusion of okadaic acid resulted in severe memory impairment, accompanied by a paired helical filament-like phosphorylation of tau protein and the formation of Aβ containing plaques-like structures in grey and white matter areas. These findings have not been, or have been only partly replicated in some laboratories (see ref. in Arendt et al., 1998). Therefore, the potentiality of this interesting model needs still to be fully explored. Moreover, no drug has been yet tested on this model with the aim to prevent or restore the damage induced by phosphatase inhibition.

Conclusions

In order to overview the preclinical studies on AD therapy, an attempt has been made to marshal the experimental models that have been used for demonstrating potential therapeutic activity and their rationals. It appears that preclinical studies have aimed mostly to understanding the pathogenic mechanisms of the disease than to testing new drugs; a correct approach, since knowledge of pathogenic mechanisms is the basis for the development of new drugs. The question arises whether the preclinical studies have really been instrumental for the development of the existing drugs. Only ChEI are so far recognised as effective in the symptomatic treatment of AD and there is little doubt that their development has been the result of four decades of preclinical studies. This long period of time saw also many failures while many drugs are still in a sort of limbo. Among the failures we count choline, phosphatidylcholine and other choline derivatives, physostigmine, and muscarinic agonists (Sarter et al., 1992). For these drugs, preclinical results were at least partly positive, but they were not substantiated by adequate demonstration of clinical efficacy. The list of drugs in the limbo includes the ampakines, phosphatidserine, Ginkgo biloba extracts, CDP-choline, calcium antagonists, and vitamin E. For them evidence obtained from clinical trials are not yet fully convincing and preclinical investigations on their mechanism of action may still be necessary.

If we take into consideration the perspective of AD therapy, the ground has been broken up by preclinical studies for drugs preventing Aβ deposition or its β-fibrillar conformation. Aβ immunization and β-sheets breakers, mentioned in previous paragraphs (Harkany et al. 1999, Schenk et al., 1999), β or γ-secretase inhibitors (Haske et al., 2000) are the most promising approaches but they still need years of preclinical and clinical studies before reaching the pharmacy shelves. The ongoing animal studies leave
also room for other therapeutic approaches such as anti-inflammatory agents, antioxidants, steroid hormones. Conversely, they seem to have ignored completely the neuropsychiatric symptoms associated with AD. It appears that no animal models of these symptoms exist. As a consequence, no attempt have been made to demonstrate whether they are related to Aβ plaque formation, cholinergic or other neurotransmitter deficits, no therapeutic proposals have been put forward, beside the symptomatic use of the usual psychotropic drugs. Is there no room for preclinical studies on this so important and distressing component of AD symptomatology?

Finally, two questions for bringing to a close this overview. Could the present level of information on AD have been reached without preclinical studies? Could our knowledge on AD be further extended, and new drugs developed without preclinical studies? I hope to have convinced the reader that the answer should be yes and no, respectively.

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