ALZHEIMER'S MEETING
REPORT

The Society for Medicines Research (SMR) held a meeting on Alzheimer's Disease at the National Heart and Lung Institute, London on 18 September 1997. This topic has come in for a lot of attention recently, partly because Pfizer have just begun marketing a new anti-cholinesterase treatment, Aricept (donepezil). This year also marked the publication of the original case notes of the first patient of Dr Alois Alzheimer to be diagnosed with a form of dementia that subsequently became known as Alzheimer's disease (Figure 1). Lastly, in recent times, a great deal has been accomplished from the viewpoint of the basic science and in particular in terms of molecular biology of the disease, and it was to review these developments that the meeting was held.

The meeting began with an overview of the neuropsychology of memory from Prof. John Hodges (Addenbrooke's Hospital, Cambridge). Memory is conventionally divided into 3 categories, namely working, episodic and semantic. Working memory is the term applied to the aspect of memory responsible for the recall of information immediately after it has been presented, and is normally associated with a location in the dorsolateral prefrontal cortex. Working memory is relatively well preserved in Alzheimer's. However, it has been shown that the recall of information (for instance thirty minutes after hearing the reading of a story) is profoundly impaired, even in the minimal AD group (Figure 2).

Episodic memory refers to personal experiences, context and time-specific events. The structures associated with episodic memory include the hippocampus and its surrounding region which is often substantially damaged, and the main area of deficit, in Alzheimer's. Semantic memory refers to our general word, concept and object knowledge, which gives meaning to our perceptual experiences. Semantic memory, which is at least partly localised to the temporal lobe, mainly to the left, is also often deficient in Alzheimer's disease.

Alzheimer's is the commonest cause of dementia, its prevalence doubling every 5 years above the age of 65. Memory impairment is the most pervasive neuropsychological deficit. There is agreement that encoding of new memories is profoundly impaired in Alzheimer's, but questions still remain as to whether there is also loss of information from stored memory. Early diagnosis of Alzheimer's is difficult, and it is worrying that standard screening instruments such as the Mini-mental State Examination (MMSE) are not sensitive detectors of the early manifestations of the disease. A more accurate measure is obtained by asking subjects to recall a story that has been recently related to them. Advances in scanning technology provide a clue to the anatomical changes that accompany AD and enable the differentiation of this type of dementia from semantic dementia (Figure 3). Beyond the episodic memory deficits that accompany early Alzheimer's, later stages of the disease involve working and semantic memory, and ultimately global intellectual breakdown accompanied by changes in behaviour and personality. However, Prof. Hodges emphasised that patients often present with atypical Alzheimer's, involving for instance spatial disorientation without memory loss.

One of the central theories of the pathophysiology of Alzheimer's that has evolved over a number of years concerns the plaques that are observed in diseased brains, and are associated with disease onset. Prof. Gareth Roberts (Opine Consultancy) gave an overview of the genetics and biochemistry of the generation of plaques, which are produced from ß-amyloid protein (ßA4), which has been shown to be neurotoxic. ß-amyloid derives from amyloid precursor protein (APP) which has been sequenced over 10 years ago. Although its function is still unknown, its structure contains a transmembrane region. There are 3 possible cleavage sites, α, ß and γ; ß and γ secretase sites

produce amyloid protein whereas the α-site does not, and this cleavage is enhanced through activation of $5HT_{2A}$ and M1 muscarinic neurotransmitter pathways. This mechanism may be important in the therapeutic effect of acetyl cholinesterase inhibitors. ß-amyloid can be formed as ßA4 (1-42) or ßA4 (1-40): the former, longer protein is more able to deposit plaques. One of the mechanisms by which ßA4 (1-42) can be enhanced is through mutations in the presenilin gene, which is perhaps a chaperone protein for ß-amyloid. In particular, presenilin-1 is associated with a serine protease (PSP-1) which is being closely looked at by SmithKline Beecham and other companies as a potential therapeutic target.

Another of the current theories in the pathophysiology of Alzheimer's is that the neuronal loss is a consequence of a loss of a functional cytoskeleton due to the accumulation of paired helical filaments (PHF). A major role in this transformation is performed by tau microtubule associated protein. In this transformation, from a normal cytoskeleton to the tangles seen commonly in biopsy tissue from Alzheimer's brains, tau is phosphorylated. Prof. Brian Anderton (Institute of Psychiatry, London) reviewed the importance of tau in Alzheimer's, showing the work that his group have conducted on the kinases that are involved in tau phosphorylation. These kinases include glycogen synthase-3 (GFSK-3) and some of the MAP kinases; GSK-3 is a physiological regulator of tau, and given its association with pathways related to presenilin, it is a plausibly important mechanism in the neurodegeneration process. However, the biochemical mechanisms underpinning both tangle and plaque formation are still incompletely understood. Despite substantial recent advances in this field, a unifying hypothesis to link plaques, tangles and dementia is still some way away.

One of the most important findings of recent years in the genetic predeterminants of Alzheimer's is the revelation by Dr Allen Roses' group that the possession of an allele for apolipoprotein E4 (apoE4) is associated with increased risk for the disease. Thus, while 0.15% of the normal population are homozygous for apoE4, this rises to 0.45% of these with AD. Put another way, 60% of apoE4 homozygotes have Alzheimer's by the age of 70. This work was summarised by Prof. Warren Strittmatter, from the group that made the discovery (Duke Univ. Med. School, North Carolina). He emphasised that apoE4 was neither necessary nor sufficient for the disease, and that apolipoprotein has other functions in the periphery, where for instance the apoE2 allele is associated with a greater risk for atherosclerosis. In the brain, apolipoprotein is involved in cholesterol distribution, but in addition is involved a number of other processes that have potential importance in neuron function. Thus, apolipoprotein regulates neurite development, binds tau protein (*in vitro*), alters tubulin metabolism and enhances tau-promoted tubulin polymerisation (*in-vitro*). In addition, apolipoprotein forms a hererodimer with the pleiotrophic factor CNTF and may therefore protect neurons from injury. These mechanisms may be relevant to the development and progression of Alzheimer's disease, however more data are required to substantiate one or more of these hypotheses.

The next speaker, Dr Palmer reported on the neurobiology of AD and the prospects for therapy. AD is the most common reason for elderly people requiring nursing home care. In addition to clinical need in AD the cost burden is some $80 bn per year is spent in the US, and if the need for nursing home care was delayed by effective AD therapy this could produce considerable savings to the healthcare system. A key question in AD is what initiates and maintains the cascade of neurodegeneration? The focus of the presentation was on excitotoxicity and the evidence for different neurotransmitters involved. Noradrenaline and 5-HT levels and uptake are both reduced in antemortem studies (~3.5 years after symptom onset). It is possible that this explains the symptoms of depression seen in sub-sets (20-30%) of AD and is a rationale for use of antidepressant drugs affecting the noradrenaline (NA) and 5-HT pathways.

Another current hypothesis of AD concerns the importance of the cholinergic nerve deficit. Evidence for this comes from reduction in synthesis of acetyl choline (ACh) (via choline acetyl transferase, ChAT) in the temporal cortex of AD post- and ante-mortem. In addition, cholinomimetics given to animals produce some of the behavioural symptoms analogous to AD. In terms of therapy, a number of AChE inhibitors are registered for use in AD. Cognex (tacrine) has been given to over 250,000 patients so far, but is limited in its use because of concerns about hepatotoxicity. It is hoped that second generation inhibitors such as Aricept (donepezil) and Exelon (rivastigmine), will be effective and have reduced side effects.

Excitatory amino-acids (EAAs) (glutamate and aspartate) may play role in AD. Changes in levels of these have been measured in AD. They may be directly neurotoxic but also by indirect pathways as they increase reactive oxygen species and thus oxidative stress. Several EAA antagonists are in the clinic, e.g. for acute brain injury, but side-effects are likely to be problematic, with psychosis, hypotension and tachycardia having been cited as of real concern. These effects would severely limit their chronic use in AD.

The subject of animal models for AD was dealt with by Dr Dora Games (Athena Neurosciences) who described the development of a transgenic mouse model which overexpresses the mutant human beta amyloid precursor protein (hAPP). The protein is has a valine to phenylalanine mutation at position 717. The animals develop a number of pathologies characteristic of AD. In both heterozygotes and homozygotes, beta amyloid protein (A_{beta}) plaques are formed in cingulate and frontal cortices and the hippocampal region. Most work has been done to date on heterozygotes. For example, the rate of A_{beta} protein deposition in the hippocampus starts at 7-9 months after birth and reached 15% by area. The homozygotes currently are producing similar pathologies, except that they occur earlier — plaques form 2 months earlier and produce 10 times greater pathology. Athena are looking at approaches to reduce A_{beta} protein and inflammation in these transgenic models.

One of the possible therapeutic strategies for AD, based on the known cholinergic deficit in the disease involves the use of cholinergic agonists to treat both behavioural and cognitive symptoms. Dr Ward described recent work at Lilly (USA) to produce selective muscarinic agonists. Muscarinic receptor subtypes M1 to M5 are expressed in brain but also in other tissues; the even numbered subtypes are negatively coupled to adenylyl cyclase, and the odd numbered subtypes to phosphoinositol. Lilly's focus was on using heterocyclic bioisosteres of the ester in arecoline, and in particular they investigated 1,2,5-oxadiazoles and 1,2,5-thiadiazoles. The optimal analogue in terms of potency and receptor efficacy was selected, and named Xanomoline (Figure 4). This had most affinity for M1 and M4 receptors, and displayed some affinity at sigma receptors. Six-month clinical trials using a 75 mg t.i.d. dosing regimen showed improvements in various standard tests of cognitive and behavioural functions. Interestingly, psychotic symptoms also appeared to be improved.

The limitations of xanomeline derive from its substantial first-pass metabolism after oral dosing, and in addition, a poor GI side effect profile which limited the doses which could be given orally and was responsible for a high dropout rate. Transdermal patch delivery is currently being trialled to obviate these side effects. A new thiadiazole analogue with selective agonistic effects against M2 and M4 receptors is under investigation. It blocks immediate firing of dopamine receptors in A10 versus A9 regions of the CNS, although without any direct affinity for dopamine (DA) receptors itself.. Animal model data suggest it has the potential as an antipschycotic without extrapyramidal side effects normally associated with DA antagonists.

There is clinical evidence that brain inflammation and oxidative stress contribute to the pathology of Alzheimer's disease. Large epidemiological studies have shown that there is a lower incidence of the disease in patients receiving non-steroidal anti-inflammatory drugs (NSAIDs). Furthermore, a recent study has also shown that the anti-oxidant Vitamin E has some beneficial effects. Dr. Julie Barnes (Glaxo Wellcome Medicines Research Centre, Stevenage) and her colleagues have been exploring the possible mechanisms underlying these clinical findings. They have shown that microglial cells, the resident phagocytes of the brain, although normally quiescent, can be activated by either lipopolysaccharide (LPS) or ß-amyloid peptide (1-40). This results in up-regulation of the inducible form of cyclooxygenase, COX-2, to produce PGE2 plus up-regulation of the inducible form of nitric oxide synthase (iNOS) producing nitric oxide (NO), which can be assayed as nitrite. TNF is also produced. The prostanoid can be inhibited by NSAIDs such as indomethacin as well as selective COX-2 inhibitors. Selective iNOS inhibitors can completely suppress the production of NO. The pathways for the production of NO, PGE2 and TNF appear to be independent.

Recent reports suggest that scavenger receptors and receptors for advanced gylcation end products (RAGE) mediate adhesion of microglia to ß-amyloid. Structure activity relationships suggested that both ß-amyloid peptides, the long form containing amino acids (1-40) or the shorter form (25-35) interact with RAGE. However, Dr. Barnes' studies show that only aggregated ß-amyloid (1-40) and not the shorter (25-35) fragment is able to activate microglia. The argument as to whether these inflammatory mediators contribute to neuronal dysfunction and cell death is not yet resolved. Dr. Barnes suggested that possible drugs of the future may include: COX-2 inhibitors; iNOS inhibitors, RAGE inhibitors, IL-l/TNF receptor antagonists and antioxidants.

Dr. Sirinathsinghji (Merck, Neuroscience Research Centre, Terlings' Park) described studies using knock-out mice designed to determine whether certain gene mutations could lead to loss of function. Mutations in three genes: presenilin 1 (PS1) and presenilin 2 (PS2), and amyloid precursor protein (APP) which are located on chromosomes 1, 14 and 21, respectively have been identified as causal agents in early onset familial Alzheimer's Disease (FAD).

Mice deficient in APP or PS1 have been generated in the Merck laboratories by homologous recombination in embryonic cells. The APP-null mice were viable and fertile and developed slowly but showed age-related neuronal dysfunction. Deficits in learning and memory and long term potentiation were observed with profound loss of synaptic markers in both, the cortex and hippocampus. Dr. Sirinathsinghji's data suggest that APP may play a critical role in the maintenance of neuronal homeostasis and synaptic function during ageing. There are several possible explanations: neurones which accumulate APP may disintegrate to form "ghost-like" structures; plaques may begin with abnormal accumulation of APP in neurons leading to disintegration and neurodegeneration may result from abnormal APP processing or back up of APP transport in neurites.

The PS1-null mutation resulted in lethality from haemorrhages in the CNS. Embryos showed defects in somatogenesis which were reminiscent of somite segmentation defects seen in mice with

functionally inactivated Notch 1 and Dll 1 (delta-like gene 1), a vertebrate Notch 1 ligand. The embryos also showed defects in sclerotome differentiation in that they exhibited abnormalities in segmentation of the axial skeleton and dorsal root ganglia. Additional data indicated impairment in neurogenesis and massive neuronal loss. These data together indicate that the PS1 gene is essential for mouse embryonic development, formation of the axial skeleton and in neurogenesis and neuronal survival. However, the function(s) of PS1 during maturation and ageing is presently unclear.

The final speaker of the day, Professor Ruth Itzhaki (Molecular Neurobiology Laboratories, UMIST, Manchester) indicated that viruses may be a possible factor in Alzheimer's disease since a number of persistent viruses cause neurological disease. In association with the Frenchay Hospital, Bristol, examination of the post-mortem brains from many aged normal and AD patients revealed the presence of latent herpes simplex type 1 virus (HSV1), as detected by sensitive PCR techniques. The argument for the involvement of HSV-1 is persuasive for the following reasons (i) its propensity for latently infecting neuronal cells; (ii) its ubiquity and (iii) its effects after acute infection of precisely the CNS regions which display the main pathological changes in AD. Two facts mitigate against this hypothesis. Firstly, AD is not transmissible and secondly most previous studies did not detect viral DNA in the CNS. However, these studies were done using Southern or dot-blotting or *in situ* hybridisation. The epsilon 4 allele of apolipoprotien E (ApoE) has been recognised as a risk factor in AD although the mechanism by which it imparts this risk is not fully understood. Examination of the ApoE genotypes of 46 AD patients and 44 age-matched (age range 54-96) normals, using PCR for HSV-1 and for genotyping, found the ApoE-4 allele frequency of AD patients to be 53% in those who were HSV-1 positive in brain and 10% in those who were HSV-1 negative. In the normal group, the frequencies were 4 and 6%, respectively. This provided evidence that possession of an E4 allele plus HSV-1 in brain confers a strong risk factor in developing the disease whereas each individual factor alone is not enough to do so. Professor Itzhaki's results seems to raise the possibility of prevention of AD by immunisation and possible retardation of its progression by anti-viral therapy. These results raise an important new theory of AD which needs to be replicated independently, but if repeated could represent a major advance in the understanding of the disease.

In summary, this meeting provided an interesting review of the recent research findings in AD, and from a multidisciplinary perspective discussed the many mechanisms which may be involved in this complicated and debilitating disease. These advances in the basic science of AD have yet to manifest themselves in terms of a breakthrough therapy, but nevertheless the recent introduction of newer, safer acetylcholine esterase inhibitors are a first step along this path.

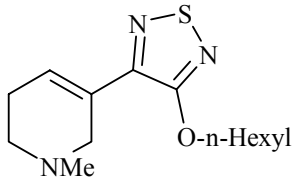

Figure 4: Xanomeline