Glutamatergic neurotransmission in Alzheimer's disease

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Introduction

Alzheimer's disease is an irreversible, neurodegenerative disorder that is characterized clinically by progressive dementia and global cognitive deterioration. Accurate diagnosis of the disease depends upon the presence of large numbers of neurofibrillary tangles (NFTs) and neuritic plaques (NPs) in the cerebral cortex and hippocampal formation. NFTs accumulate in the perikarya of affected neurons, whereas NPs represent the abnormal foci of degenerating nerve endings [1].

The pattern of neurotransmitter deficits has been extensively studied in Alzheimer's disease brain. Studies on both biopsy and frozen post-mortem material have established losses of cortical cholinergic, serotonergic and noradrenergic innervation from subcortical nuclei [2]. However, it is the neuronal degeneration within the cerebral cortex that is characteristic of Alzheimer's disease.

In addition to NFTs and NPs in the cerebral cortex and hippocampal formation, there is evidence of loss of cortical neurons, particularly the large pyramidal cells. This loss of cortical pyramidal neurons has been shown to correlate with the clinical severity [3] and progression [4] of the disease, suggesting that cortical pyramidal neuron dysfunction plays an important part in the disease process. Further evidence suggesting the importance of pyramidal neuron dysfunction in Alzheimer's disease has come from studies demonstrating that cortical NFTs are concentrated in the pyramids of neurons of layers III and V which provide the major cortico-cortical and cortical efferent projections [5, 6].

Glutamatergic neurons in Alzheimer's disease

Animal studies have demonstrated that pyramidal neurons of the cerebral cortex and hippocampal formation use glutamate or a related amino acid/small peptide as a neurotransmitter [7]. However, the biochemical correlate of pyramidal neuron dysfunction in Alzheimer's disease has been difficult to establish owing to the lack of a suitable marker for glutamatergic neurons in post-mortem human brain. Glutamate levels are reported to be relatively stable post mortem [8] and a number of workers have reported reduced levels of free glutamate in Alzheimer's disease cerebral cortex and hippocampal formation [9-11]. Similarly, reduced levels of aspartate have been reported in Alzheimer's disease [11]. However, measurements of this amino acid may be complicated by the rapid rise in levels that occurs within a few hours after death [8]. In addition to its role as a neurotransmitter, glutamate is important as an intermediary metabolite and measurements of free glutamate levels do not distinguish between transmitter and metabolic pools of this amino acid. Furthermore, measurements of free amino acid levels may be complicated by an accelerated proteolysis in Alzheimer's disease compared with control subjects [12]. In view of the problems outlined above, we decided to develop an alternative strategy for determining the integrity of glutamatergic neurons in Alzheimer's disease human brain.

The tracing of glutamatergic pathways in animal brain has largely been achieved by measuring glutamate uptake after selective lesioning, as well as by following the retrograde transport of labelled amino acids after uptake by intact nerve terminals [13]. In view of these studies, we decided to investigate the possibility of using amino acid uptake as a marker of glutamatergic terminals in post-mortem human brain. This approach was stimulated by previous work demonstrating the feasibility of preparing metabolically active synaptosomes from suitably frozen post-mortem human brain material [14, 15].

In a comparison of rat and human brain, we demonstrated that synaptosomal uptake of the stable glutamate analogue, D-aspartate, occurred by a similar process in both species. D-aspartate uptake into synaptosomes prepared from previously frozen post-mortem human brain showed similar kinetic profiles, sodium dependence and pharmacological characteristics as those seen in fresh rat brain [16]. Furthermore, although uptake into rat brain preparations was reduced by freezing, the effects of post-mortem delay were minimal [17, 18].

Having demonstrated the feasibility of using synaptosomal D-aspartate uptake as a marker for glutamatergic terminals in previously frozen post-mortem human brain, we went on to determine the effects of Alzheimer's disease on this process. In a comparison of D-aspartate uptake into synaptosomes from Alzheimer's disease and control subjects that had been matched for synaptosomal respiratory rate and subject post-mortem delay, age and sex, we demonstrated significant losses of apparent glutamatergic terminals in preparations from the cerebral cortex and hippocampal formation of the disease group. Importantly, no significant reductions were seen in subcortical areas that typically show little pathology in the disorder [17]. This finding of an apparent loss of glutamatergic terminals in Alzheimer's disease has been shown by others using measurements of D-aspartate uptake into fresh tissue, thus confirming that the observed deficits are not a result of tissue freezing [19].

The comparison of active processes in Alzheimer's and control subjects has been criticised owing to the possible influence of metabolic dysfunction and agonal status in Alzheimer's disease brains [19]. In view of this, we felt it important to confirm the above findings using an alternative assay that would not be complicated by possible metabolic abnormalities. The confirmation of the loss of glutamatergic terminals in Alzheimer's disease brain has been achieved by ourselves and others using extensively washed and lysed membrane preparations and sodium-dependent D-aspartate binding as a marker [20]. Significant reductions in D-aspartate binding have been reported in the temporal cortex, hippocampal formation and caudate nucleus of Alzheimer's disease subjects [21-25]. It is important to note from these studies that the temporal lobe and hippocampal formation typically show the most severe pathology in Alzheimer's disease. Furthermore, histological studies of biopsy material from Alzheimer's disease temporal lobe have demonstrated significant losses of synapses per surviving pyramidal neuron, thus providing a histopathological correlate of the glutamatergic deafferentation observed in this region [26].
Glutamate receptor integrity in Alzheimer's disease

The post-synaptic actions of glutamate are mediated by three distinct receptor subtypes that have been characterized on the basis of the actions of the prototypic agonists N-methyl-D-aspartate (NMDA), kainate and quisqualate [27]. The most extensively characterized glutamate receptor is the NMDA subtype. Agonists acting at this receptor generate responses using an ion channel-dependent manner that can be blocked non-competitively by Mg$^{2+}$ and Zn$^{2+}$ as well as by several organic molecules that interact with non-$\sigma$ phenocyclidine (PCP) binding sites. The latter include PCP, ketamine and (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclopepten-5,10-imine maleate (MK-801), which are believed to act at the level of the NMDA-receptor-associated ion channel. In contrast, augmentation of NMDA-induced responses can be achieved with glycine, which acts at a site distinct from the agonist recognition site or from those sites which bind either Mg$^{2+}$, Zn$^{2+}$ or the non-competitive antagonists [28]. NMDA receptors have been implicated in the generation of long-term potentiation and excitotoxic cell death. The former is believed to represent an important mechanism for information storage and memory formation in the mammalian brain [29] and NMDA receptor antagonists have been shown to block spatial learning processes in the rat [30]. In addition, glutamate excitotoxicity has been suggested to be important in the pathogenesis of a number of neurological disorders, including Alzheimer's disease [31, 32]. For these reasons, we felt it important to determine the integrity of NMDA receptors in the disorder.

Alzheimer's disease may represent an important factor in determining the extent to which the findings of the number of other studies that have used assays specific for the ion-channel-associated, non-competitive, antagonist binding site [23, 34–36]. However, it should be noted that other studies have reported a loss of NMDA receptors in Alzheimer's disease [37–39]. The extent to which the findings of the latter studies are owing to the different assay procedures used or the severity of the disease process in the cases studied remains to be established [40].

An alternative explanation for the discrepancy between the findings of different studies of NMDA receptors in Alzheimer's disease may be the existence of distinct subtypes of the receptor. Of relevance to this, is the recent report of agonist-and antagonist-prefering classes of NMDA recognition sites with differential anatomical distribution and regulation by glycine [41]. Also of relevance are the recent reports of a deficit in the glycine recognition site of the NMDA receptor in Alzheimer's disease [42, 43]. Although these studies require independent confirmation, it is tempting to speculate that any NMDA receptor dysfunction in Alzheimer's disease may represent a subtle abnormality in one component of the NMDA receptor complex which leads to a loss of a particular conformational form of the receptor.

Summary

The studies described here provide convincing evidence of glutamatergic deafferentation in Alzheimer's disease. This deafferentation is presumed to represent the biochemical correlate of pyramidal neuron dysfunction in the disorder. In addition, there is a growing consensus of opinion that there is no widespread depletion of glutamate NMDA receptors in Alzheimer's disease. The significance of glutamatergic neurotransmission abnormalities to the pathogenesis and clinical progression of the disease remains to be established.

Alzheimer’s disease (AD) is a human cerebral degenerative disorder characterized by a deterioration in cognitive function, loss of memory and decreased ability to learn new information or acquire new skills.

At the cellular and molecular level, the cardinal histopathological findings of AD are neurofibrillary tangles (NFT) and senile plaques (SP) [1, 2]. These features reflect either cell death or at least extreme dysfunction of selected populations of neurones. SP are composed of a material generically called amyloid at the centre of which frequently lies an aluminosilicate complex [3]. NFT are primarily composed of paired helical filaments (PHF), two helically wound 10 nm diameter fibres [4]. The exact molecular nature of the PHF remains unknown, but immunocytochemical studies have revealed similarities between PHF and some normal cytoskeletal proteins such as the microtubule-associated protein-tau (MAP-tau) subunits, microtubule-associated proteins (MAPs) and microtubule-associated protein-tau (MAP-tau) [5, 6]. Of several theories advanced to explain the pathogenesis of SP and NFT in AD, the idea of a neurotoxin, particularly aluminium, has aroused most interest. Aluminium has been found associated with both SP [3] and NFT [7] in AD brain specimens and has also been implicated as the actiological agent in dialysis encephalopathy syndrome [8] and other dementias resembling AD.

The use of animal models to study aluminium uptake has revealed little information on the amount of aluminium entering cells or on the perturbations of the normal neuronal cytoskeleton leading to the development of NFT and SP. Equally, in the few previous studies which have investigated the effects of aluminium on cell cultures [9–11], conditions of treatment have been ill defined and the levels of the metal ion inside the cells were not measured. Failure to glean information in both the studies in vivo and in vitro to date has been due mainly to the insoluble nature of the aluminium salts used.

On this basis, we developed a model in vitro using human neuroblastoma cells (which show many of the characteristics of normal neurones) and soluble complexes of aluminium in an attempt to measure precisely the intracellular levels of aluminium. We are also using the system to study changes in the cytoskeleton via immunocytochemical and biochemical techniques.

In our experiments, human neuroblastoma cells were exposed to a range of concentrations of aluminium (0–2.5 mM), in Eagle’s minimum essential medium, when complexed to one of three ‘carrier’ molecules, namely EDTA, citrate or 3-hydroxy-2-methyl-4H-pyran-one (maltol). After a 1-week incubation period, cells were harvested and counted. Both culture medium and cells were then analysed for aluminium content by atomic absorption spectroscopy.

For immunocytochemistry (in collaboration with D. M. A. Mann & D. Jones), cells were grown on poly-L-lysine-coated slides and exposed to culture medium containing aluminium–EDTA for varying periods of time. Slides were then ‘stained’ using antibodies raised against certain cytoskeletal components, namely tau protein and PHF. In both cases (i.e. for measurement of aluminium uptake and for immunocytochemistry), control cells were exposed to culture medium alone and culture medium containing carrier.

Our results comparing the uptake of aluminium into neuroblastoma cells while complexed to various ‘carrier’ molecules show that uptake was low in all cases apart from aluminium–EDTA (Fig. 1). After treatment with the latter, the aluminium in the cells initially increased with increasing level of aluminium–EDTA in the culture medium, but eventually reached a plateau at two to three times that of cells treated with EDTA alone. Calculation of the aluminium con-