

Potential role of *N*-methyl-D-aspartate receptors as executors of neurodegeneration resulting from diverse insults: focus on memantine

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Glutamatergic neurotransmission is critical to normal learning and memory and when the activity of glutamate neurons becomes excessive, or the normal function of its primary receptors becomes dysfunctional, this may lead to pathological changes associated with age-related neurodegenerative diseases. Anomalous glutamatergic activity associated with Alzheimer's disease may be due to a postsynaptic receptor and downstream defects that produce inappropriately timed or sustained glutamate activation of *N*-methyl-D-aspartate receptors, leading to neuronal injury and death and cognitive deficits associated with dementia. The mechanisms leading to the condition of chronically depolarized membranes on vulnerable neurons in the Alzheimer's disease brain are likely due to a complex interaction between oxidative stress, mitochondrial failure, chronic brain inflammation and the presence of amyloid- β and hyperphosphorylated-tau; each of these factors are

highly interrelated with each other and are discussed with an emphasis upon potential therapeutic mechanisms underlying the neuroprotective actions of memantine. *Behavioural Pharmacology* 17:411–424 © 2006 Lippincott Williams & Wilkins.

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Introduction

Although glutamatergic neurotransmission is critical to normal learning and memory, when the activity of glutamate neurons or the stimulation of glutamate receptors becomes dysfunctional, this may lead to well-characterized pathological consequences, particularly associated with age-related neurodegenerative diseases. Evidence exists suggesting that the anomalous glutamatergic activity associated with Alzheimer's disease (AD) may be due to a postsynaptic receptor and/or a downstream defect. Particular importance has been attributed to inappropriately timed or sustained glutamate activation of *N*-methyl-D-aspartate (NMDA) receptors leading to neuronal injury and death (Greenamyre *et al.*, 1988; Mattson *et al.*, 1993; Dodd *et al.*, 1994; Holscher, 1998). In addition, the same dysfunction may cause cognitive deficits associated with dementia (Danysz and Parsons, 2003).

Glutamatergic neurotransmission is mediated through ionotropic glutamate receptors such as α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, kainate and NMDA receptors (Parsons *et al.*, 1998). Additionally, glutamate activates G-protein coupled metabotropic glutamate receptors that are believed to have a more modulatory function (Pin and Acher, 2002). Most α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors are impermeable to Ca^{2+} and contribute to fast

synaptic transmission. In contrast, NMDA receptors are characterized by high permeability to Ca^{2+} ions, voltage-dependent blockade by Mg^{2+} ions and slower gating kinetics. At normal resting potentials, the transmembrane electric field (negative on the inside of the cell) favours entry of positively charged Mg^{2+} into the pore of the receptor so that the channel is blocked. Under such resting conditions, NMDA receptors do not conduct ions. With sufficient postsynaptic depolarization within the neuronal membrane surrounding the receptor however, Mg^{2+} is no longer strongly attracted into the pore of the channel and dissociates. Under such depolarized conditions, NMDA receptors activated by synaptically released glutamate allow the influx of Na^+ and, in particular Ca^{2+} , contribute to postsynaptic excitation and activation of second messenger systems. These features make NMDA receptors quite suitable for mediating plastic changes in the brain, such as learning (Morris *et al.*, 1986; Collingridge and Singer, 1990; Danysz *et al.*, 1995; Zajaczkowski *et al.*, 1997).

These features, however, may also contribute to the neurotoxicity owing to Ca^{2+} overload. Namely, upon mild depolarization, magnesium block is removed and NMDA channels remain open (permeable to Ca^{2+} and other ions) until the agonist – glutamate – is removed. Under these conditions, ambient levels of glutamate can activate NMDA receptors allowing excessive calcium ion influx

which, if sufficiently prolonged, may trigger a cascade of events leading to neuronal injury and death (Choi *et al.*, 1987). Thus, although neurons can cope with short-lasting high influx of Ca^{2+} , seen during physiological activation, the compensatory mechanisms are not sufficient in case of lower, but prolonged influx as likely occurring in acute or chronic neurodegeneration. The mechanisms leading to the condition of chronically depolarized membranes on vulnerable neurons in the AD brain may be due to a complex interaction between oxidative stress, mitochondrial failure, chronic brain inflammation and the presence of amyloid- β ($\text{A}\beta$) and possibly hyperphosphorylated-tau. Each of these factors is highly interrelated with each other and will be discussed below. Importantly, the cellular dysfunction is likely to be entirely postsynaptic as elevated ambient glutamate levels have not been observed in the AD brain. Owing to the critical involvement of NMDA receptors in these processes, we hypothesize that an NMDA receptor antagonist should show efficacy in the treatment of AD. The NMDA antagonist 1-amino-3,5-dimethyladamantane (memantine) was recently registered in multiple countries for treatment of patients with moderate-to-severe to severe AD. Hence, memantine offers a unique tool to verify clinically the findings obtained from animal experiments and therefore is the focus of the current review. Given our current understanding of the multiplicative role of the NMDA receptors in numerous disease states, we propose that memantine, and possibly other NMDA antagonists with similar features, should provide significant clinical benefits in a variety of disorders (see Sonkusare *et al.*, 2005).

Memantine as an *N*-methyl-D-aspartate receptor antagonist

The first publication describing memantine appeared in 1963 by researchers at Eli Lilly (Gerzon *et al.*, 1963); however, the first demonstration of its actions in the central nervous system was made in 1972 by Merz and Co., who applied for a German patent (see Table 1). Initially, memantine was considered for the treatment of Parkinson's disease and spasticity and also for cerebral disorders such as coma, cerebrovascular and age-related psychiatric disturbances (Grossmann and Schutz, 1982; Miltner, 1982a, b; Schneider *et al.*, 1984; Munding and Milios, 1985). The initial effects of memantine in animal studies resembled dopaminomimetics (Maj, 1982; Wesemann *et al.*, 1983); however, these effects were only observed at plasma levels that were much higher than those achieved in patients with AD (Danysz *et al.*, 1997; Parsons *et al.*, 1999). Memantine was shown to be a NMDA receptor antagonist in 1989 (Kornhuber *et al.*, 1989); a finding later confirmed by others (Chen *et al.*, 1992; Parsons *et al.*, 1993). Clinical studies confirmed the effectiveness of memantine in the symptomatological treatment of AD and additional benefits resulting from its

combined use with cholinesterase inhibitors (Ditzler, 1991; Gortelmeyer and Erbler, 1992; Orgogozo *et al.*, 2002; Wilcock *et al.*, 2002; Reisberg *et al.*, 2003; Tariot *et al.*, 2004; Gauthier *et al.*, 2005; Reisberg *et al.*, 2006).

In general, published and unpublished data indicate that at therapeutic doses (typically 20–30 mg/day), at steady-state (chronic treatment for several weeks), plasma levels of memantine are in a range of 0.4–1 $\mu\text{mol/l}$ (Kornhuber and Quack, 1995; Danysz *et al.*, 1997; Periclou *et al.*, 2006). In rats, this range of concentrations is achieved either by infusion of 10–30 mg/kg/day (typically 20) using osmotic pumps, or after acute intraperitoneal injection of 2.5–5.0 mg/kg as measured at peak (15–30 min) (Danysz unpublished; Danysz *et al.*, 1994b, 1997; Misztal *et al.*, 1996; Wenk *et al.*, 1996; Hesselink *et al.*, 1999; Zolad *et al.*, 2006). Initial studies reported very high (> 20 $\mu\text{mol/l}$) brain levels of memantine after moderate doses in animals (Wesemann *et al.*, 1982), which would indeed raise a question whether NMDA antagonism is really the mechanism of action. This assessment does not, however, take into account the fact that memantine is accumulated in intracellular compartments (primarily by lysosomes) (Honegger *et al.*, 1993). Indeed, the analysis of memantine levels in brain homogenates in rats revealed 30-fold higher values than those found in either extracellular fluid (brain microdialysis) or cerebrospinal fluid (cistern magna) sampling (Hesselink *et al.*, 1999). If the maximal level of memantine is assumed to be 1 $\mu\text{mol/l}$, then any receptor that expresses an affinity of low $\mu\text{mol/l}$ or lower should be considered as a potential target. Given this assumption, there are only four plausible targets: the NMDA receptor channel, 5-HT₃ receptors and the nicotinic receptors of $\alpha 7$ or $\alpha 4\beta 2$ type (Buisson and Bertrand, 1998; Maskell *et al.*, 2003; Rammes *et al.*, 2004; Aracava *et al.*, 2005). Antagonism of the 5-HT₃ receptor does not seem to play a major role *in vivo*. Memantine (5 mg/kg) had no antiemetic effect in cisplatin-treated ferrets, in contrast to treatment with ondansetron or granisetron (Lehmann and Karrberg, 1996; Merz unpublished data) indicating a lack of 5-HT₃ blockade after behavioural active doses. Memantine can inhibit $\alpha 4\beta 2$ responses with an IC_{50} = 6.6 $\mu\text{mol/l}$ (Buisson and Bertrand, 1998); however, this level is probably too high to be of real therapeutic significance. In line with this conclusion, *in-vivo* memantine, at 10 mg/kg, had only a mild effect on nicotine discrimination (Zakharova *et al.*, 2005) mediated by $\alpha 4\beta 2$ receptors (Mansbach *et al.*, 2000), at doses that also affected saline responding, indicating lack of selectivity. The reported potency of memantine at $\alpha 7$ receptors varies considerably from 0.33–1.68 $\mu\text{mol/l}$ seen in rat receptors (Aracava *et al.*, 2005) to 5 $\mu\text{mol/l}$ observed in human receptors, making this action less likely to contribute to the therapeutic effects in AD patients (Maskell *et al.*, 2003). Recent studies (Nagel *et al.*, personal communication), however, indicate that at 5–10 mg/kg, memantine does not produce changes in

Table 1 Development of knowledge and concepts on memantine (focus on dementia and neuroprotective activity)

When	What	References
1963	First description of memantine as part of a synthetic pathway	Gerzon <i>et al.</i> (1963)
1989	Description of NMDA antagonistic activity of memantine in patch clamp experiments	Bormann (1989)
1989	Inhibition of [³ H]MK-801 binding by memantine in human postmortem cortical tissue	Kornhuber <i>et al.</i> (1989)
1990	Description of neuroprotective effects of memantine <i>in vitro</i> and <i>in vivo</i> in ischaemia	Seif El Nasr <i>et al.</i> (1990)
1991	Description of positive effects of memantine on cognition in demented patients	Ditzler (1991)
1992	Description of kinetics of NMDA channel blockade, ephemeral block suggested to be important for neuroprotective efficacy without side effects	Chen <i>et al.</i> (1992)
1992	Neuroprotection of the hippocampus against NMDA agonist (quinolinic acid) induced damage	Keilhoff and Wolf (1992)
1992	Neuroprotective effects against HIV <i>in vitro</i>	Lipton (1992); Müller <i>et al.</i> (1992)
1993	Importance of voltage dependency of memantine for clinical tolerability postulated and similarity to the endogenous NMDA antagonist Mg ²⁺ emphasized. Signal-to-noise concept as an explanation for symptomatic improvements in AD discussed for the first time.	Parsons <i>et al.</i> (1993)
1993	Review emphasizing importance of moderate affinity for tolerability of memantine and related agents	Rogawski (1993)
1994	Demonstration of behavioural differences between memantine and high-affinity NMDA channel blockers	Danysz <i>et al.</i> (1994a)
1994	Demonstration of NMDA antagonism in an <i>in-vivo</i> preparation	Herrero <i>et al.</i> (1994)
1994	Neuroprotective effect relevant for glaucoma	Osborne and Herrera (1994)
1994	Study showing free memantine levels <i>in vivo</i> close to affinity at NMDA receptors	Spanagel <i>et al.</i> (1994)
1994	Demonstration of neuroprotective activity <i>in vivo</i> relevant for Alzheimer's disease	Wenk <i>et al.</i> (1994)
1995	Data on concentration of memantine in plasma and CSF of patients	Kornhuber and Quack (1995)
1995	Comparison of a series of NMDA channel blockers emphasizing the importance of voltage-dependency and kinetics	Parsons <i>et al.</i> (1995)
1995	Neuroprotective activity against HIV <i>in vivo</i>	Toggas <i>et al.</i> (1996)
1995	Demonstration of memantine dose response in insult relevant for Alzheimer's dementia (ED ₅₀ =2.8) and quantitative demonstrating of better therapeutic index than high-affinity NMDA antagonists (like MK-801)	Wenk <i>et al.</i> (1995)
1996	Neuroprotective activity of memantine (prevention) in tardive dyskinesia model	Andreassen <i>et al.</i> (1996)
1996	Study showing that memantine may improve neuronal plasticity and learning in old rats	Barnes <i>et al.</i> (1996)
1996	Demonstration that memantine is relatively less potent in blocking neuronal plasticity (LTP) than NMDA receptors as compared with the high affinity NMDA antagonist MK-801	Frankiewicz <i>et al.</i> (1996)
1996	Memantine prevention of quinolinic acid-induced learning impairment	Misztal <i>et al.</i> (1996)
1996	Neuroprotection against mitochondrial toxins <i>in vivo</i>	Wenk <i>et al.</i> (1996); Schulz <i>et al.</i> (1996)
1996	Neuroprotection in an <i>in vivo</i> model of MS	Wallstrom <i>et al.</i> (1996)
1996	Demonstration of learning enhancement in rats with learning deficit	Zajackowski <i>et al.</i> (1996)
1997	Review describing non-published and published data on the <i>in-vitro</i> activities of memantine and relating pharmacokinetics of memantine to pharmacodynamics	Danysz <i>et al.</i> (1997)
1997	Importance of partial trapping for tolerability of memantine	Blanpied <i>et al.</i> (1997)
1997	Experimental basis for the concept that overactivation of NMDA receptors impairs neuronal plasticity (LTP) and learning which are restored by memantine, as suggested through a decrease in synaptic noise	Zajackowski <i>et al.</i> (1997)
1998	Efficacy shown in mixed vascular and Alzheimer dementia patients	Winblad and Poritis (1999)
1999	Memantine reduced injury in spinal cord ischaemia	Ehrlich <i>et al.</i> (1999)
1999	Noise reduction hypothesis of memantine symptomatological action substantiated by experiments showing restoration of synaptic plasticity (LTP) under low magnesium concentrations	Frankiewicz and Parsons (1999)
1999	Quantitative analysis of relation between memantine plasma, CSF, ECF and brain homogenates concentration using <i>in-vivo</i> recovery	Hesselink <i>et al.</i> (1999)
1999	Review describing pharmacology of memantine and hypothesis on the mechanism of action	Parsons <i>et al.</i> (1999)
2000	Description of the signal to noise hypothesis explaining both neuroprotective and symptomatological effects of memantine	Danysz <i>et al.</i> (2000)
2000	Memantine shown to have better therapeutic index than MK-801 using neuroprotection against hypoglycaemia-hypoxia and LTP in hippocampal slices	Frankiewicz <i>et al.</i> (2000)
2000	Lack of negative interaction between memantine and clinically used AchEIs shown <i>in vitro</i>	Wenk <i>et al.</i> (2000c)
2000	Protective effect of memantine against inflammation in brain regions relevant for AD	Willard <i>et al.</i> (2000)
2001	Support for voltage-dependent NMDA channel blockade by memantine <i>in vivo</i>	Jones <i>et al.</i> (2001)
2001	Memantine effective in TBI	Rao <i>et al.</i> (2001)
2002	Memantine prevents neurodegeneration produced by acute injection of Aβ	Miguel-Hidalgo <i>et al.</i> (2002)
2003	Review of signal to noise hypothesis	Danysz and Parsons (2003)
2003	Study showing cognitive improvement in AD patients treated	Reisberg <i>et al.</i> (2003)
2003	Review on memantine on proposed mechanism of action (MOA)	Rogawski and Wenk (2003)
2004	Memantine prevents <i>in-vitro</i> hyperphosphorylation of tau and deficits in cell viability produced by PPA2 inhibition by okadaic acid	Li <i>et al.</i> (2004)
2004	Memantine shown to inhibit progression in Huntington's disease in a small clinical study	Beister <i>et al.</i> (2004)
2004	Study showing that memantine does not block ischaemic tolerance <i>in vitro</i>	Frankiewicz and Parsons (2004)
2004	Memantine shown to improve spatial learning in transgenic preseniline 1 (PS1/Aβ) mice	Minkeviciene <i>et al.</i> (2004)
2004	Memantine showed no change of AchE inhibition by rivastigmine and donepezil <i>in vivo</i>	Enz and Gentsch (2004); Periclou <i>et al.</i> (2004)
2005	Study showing that memantine does not block ischaemic tolerance <i>in vivo</i>	Duszczyk <i>et al.</i> (2005)
2005	Chronic memantine prevents Aβ induced impairment of delayed non matching to position (DNMTP) task	Yamada <i>et al.</i> (2005)
2005	Chronic memantine prevent cognitive decline in transgenic APP23 mice	Van Dam and De Deyn (2006)
2005	Memantine prolongs survival in transgenic model of ALS (SOD mice)	Wang and Zhang, (2005)

AD, Alzheimer's disease; CSF, cerebrospinal fluid; NMDA, *N*-methyl-D-aspartate; ECF, extracellular fluid; AchEI, acetylcholine esterase inhibitor; Aβ, amyloid-β; SOD, superoxide dismutase.

dopamine levels in the striatum that would be consistent with blockade of $\alpha 7$ receptors (Rassoulpour *et al.*, 2005). In contrast, memantine has neuroprotective activity against NMDA infused into the basal forebrain of rats at low doses, that is, with $ED_{50} = 2.8$ mg/kg (Wenk *et al.*, 1995).

Given the crucial role of NMDA receptors in neuronal plasticity, the fact that memantine improves cognition and neuronal plasticity seems at first glance paradoxical. It should be realized, however, that magnesium is an endogenous NMDA channel blocker and its removal from the channel leads both to an impairment in neuronal plasticity (Coan *et al.*, 1989; Frankiewicz and Parsons, 1999) and neuronal death (Furukawa *et al.*, 2000). Any dysfunction of postsynaptic neurons leading to weakened blockade by magnesium ions, for example owing to partial depolarization as a consequence of an energy deficit, may trigger such functional (plasticity) and structural (neuronal loss) deficits (Danysz and Parsons, 2003; Rogawski and Wenk, 2003). As memantine is more potent and slightly less voltage-dependent than magnesium, it may serve as a more effective surrogate for magnesium ions (Parsons *et al.*, 1993). As a result of its somewhat less pronounced voltage-dependency, memantine is more effective than Mg^{2+} in blocking tonic pathological activation of NMDA receptors at moderately depolarized membrane potentials. Following strong synaptic activation however, memantine, like Mg^{2+} , can leave the NMDA receptor channel with voltage-dependent, fast unblocking kinetics. In turn, memantine suppresses synaptic noise but allows the relevant physiological synaptic signal to be detected. This provides both neuroprotection and symptomatic restoration of synaptic plasticity by one and the same mechanism (Parsons *et al.*, 1999; Danysz and Parsons, 2003). Antagonists that have 'too high' affinity for the channel or 'too little' voltage dependence, such as dizocilpine [(+)-5-methyl-10,11-dihydro-5H-dibenzocyclohepten-5,10-imine maleate, (+)MK-801], do not have a favourable therapeutic profile and produce numerous side effects as they essentially act as an irreversible plug of the NMDA receptor channel and blocks both pathological and physiological function.

Thus, factors such as affinity, kinetics and voltage dependency are crucial determinants of both the effectiveness and tolerability of memantine (Parsons *et al.*, 1993, 1999; Danysz and Parsons, 2003).

The potential mechanisms underlying the neuroprotective effects of memantine have been addressed by others (Chen *et al.*, 1992; Lipton, 2006), who have suggested that memantine blocks NMDA channels in an agonist concentration-dependent manner. In this case, more receptor activation by higher concentrations of agonist

is claimed to be associated with more block by memantine. Additionally, memantine is claimed not to remain trapped in the resting channel. This hypothesis seems, to us, to be unlikely because a simplistic interpretation of such a mechanism would predict that memantine should block normal synaptic transmission more effectively than moderate pathological overactivation, as, in the former case, there is a much larger increase in glutamate concentration to millimolar levels, albeit only for several hundred milliseconds (Clements *et al.*, 1992). The two hypotheses do have one aspect in common, that is, the fact that memantine block of NMDA receptors has fast kinetics. The accepted fact that memantine is, however, a trapping and not a sequential channel blocker (Johnson and Kotermanski, 2006) is clearly at odds with the hypothesis of Dr Lipton, but fully supportive of our hypothesis, in which memantine remains trapped in the NMDA receptor channel under resting conditions and blocks it in a similar manner to Mg^{2+} , albeit with greater affinity. Additionally, this hypothesis (Lipton, 2006) does not provide an explanation for symptomatological effects seen as early as 2 weeks after treatment (Ditzler, 1991), which are unlikely to be a direct consequence of the predicted long-term neuroprotective effects of memantine.

Neuroinflammation

Chronic neuroinflammation is a probable key factor underlying neuronal death and the pathophysiologic development of AD (Akiyama *et al.*, 2000; Wenk *et al.*, 2000a). Counteracting these processes with anti-inflammatory agents has been theorized to protect against the disease. Epidemiological evidence supports the chronic use of nonsteroidal antiinflammatory drugs (NSAIDs) for reducing the risk of AD (Andersen *et al.*, 1995); however, clinical trials have produced mostly negative results. Investigators have found a significant association between exposure to NSAIDs for more than 2 years and AD risk reduction (Breitner *et al.*, 1994). Recent studies suggest that although anti-inflammatory agents do not appear to slow progression of dementia, they may have a preventive influence on the development of AD pathology (Breitner *et al.*, 1994; Wenk *et al.*, 2000a). Additional work to further evaluate their potential protective properties is warranted.

Inflammatory changes are closely related to the cognitive and neuropathological manifestations of AD (Akiyama *et al.*, 2000). In the brains of AD patients, especially within the entorhinal and frontal cortex, inflammatory markers, such as activated microglia, demonstrate a higher correlation with synapse loss than does the number of neurofibrillary tangles (DiPatre and Gelman, 1997) or the degree of deposition of $A\beta$ (Terry *et al.*, 1991). The brain of an AD patient expresses a significant and well-organized cascade of immunological changes and these

changes occur very early in the progression of the disease in brain regions that later show the greatest concentration of senile plaques and atrophy (Cagnin *et al.*, 2001).

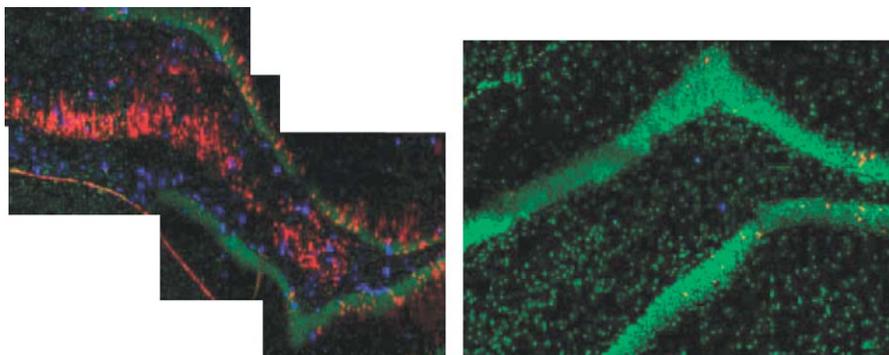
Memory impairments in the early phases of AD coincide with the development of inflammation within neuronal populations and regions known to be vulnerable in the brains of AD (Davis *et al.*, 1999). The processes underlying the commencement of the inflammation lead to a cascade of self-propagating cellular events including blockade of glutamate uptake by the glia (Rothwell *et al.*, 1997), increased release of prostaglandins (Katsura *et al.*, 1989) and enhanced release of glutamate (Hanisch *et al.*, 1997; Emerit *et al.*, 2004). Inflammation can also relieve the magnesium ion blockade of voltage-gated NMDA channels and increase nitric oxide levels, both leading to calcium ion flux dysregulation, impaired mitochondrial respiration, oxidative stress, a decline in energy production and membrane depolarization (Chao *et al.*, 1995; Emerit *et al.*, 2004). Subsequent activation of NMDA receptors by glutamatergic synaptic activity may thus permit a continuous influx of calcium ions into neurons, theoretically overwhelming the endogenous mechanisms that regulate calcium ion homeostasis (Albin and Greenamyre, 1992; Chao and Hu, 1994).

The amplitude of the calcium ion entry through NMDA channels, the kinetics of calcium ion release from intracellular stores, the decay in its free cytoplasmic levels and spatiotemporal pattern of activation of NMDA channels distributed around the neural networks within the hippocampus are the principal means by which calcium ion signals are deciphered into a meaningful biological response that can lead to the consolidation of a new memory. The consequences of long-term, low-level brain inflammation might therefore lead to a destabilization of neuronal calcium ion homeostasis and further alteration of signal-transduction cascades (Barry *et al.*,

2005), including the synaptic modifications that depend upon the expression of specific immediate-early genes, particularly the expression of *Arc* in the hippocampus following behavioural experience (Guzowski *et al.*, 1999, 2000; Guzowski, 2002). Changes in *Arc* gene expression have been connected with cognitive impairment and amyloid deposition in aged transgenic mice (Dickey *et al.*, 2004). *Arc* protein may interact with calcium-dependent intracellular second messengers (Husi *et al.*, 2000). Not surprisingly, chronic neuroinflammation led to a significant increase in the number of neurons showing exploration-related *Arc* mRNA transcription and *Arc* protein translation in hippocampal regions that also showed the greatest number of inflammation-induced activated microglia (see Fig. 1, left side) (Rosi *et al.*, 2005). *Arc* may traffic glutamate receptors in the dendritic spines of hippocampal neurons (Guzowski *et al.*, 2006) and the presence of neuroinflammation may lead to altered synaptic plasticity through an impairment of this trafficking function (Rosi *et al.*, 2005). The small proportion of *Arc*-expressing neurons in selected regions after behavioral exploration is consistent with the principle of sparse distributed coding (McNaughton and Morris, 1987), which suggests that in order to achieve efficient memory storage, only a small percentage of the total population of neurons should represent an episode (McNaughton *et al.*, 1996; Sakurai, 1999). The dramatic increase in the number of neurons expressing *Arc* in response to the chronic inflammation likely disrupts sparse coding and decreases the plasticity of the system. These changes in *Arc* expression may represent disrupted information processing associated with impaired learning and memory abilities (Rosi *et al.*, 2005, 2006).

NMDA receptor dysregulation was most evident within brain regions showing the highest degrees of inflammation. We speculate that the general neuronal dysfunction that develops as a consequence impairs the mechanisms

Fig. 1



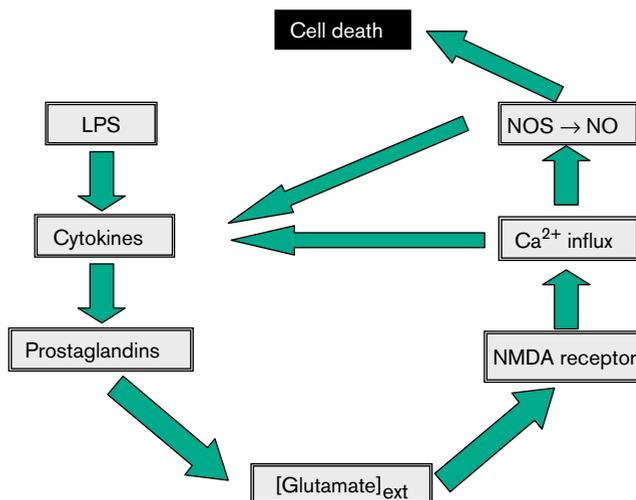
Immunofluorescence staining for the *Arc* protein (red) and activated microglia (blue) within the Dentate gyrus (DG) of young rats infused with lipopolysaccharide and treated with either the vehicle (left side) or memantine (15 mg/kg/day, right side) 90 min after exploration experience. Nuclei are counterstained in green.

underlying synaptic plasticity, such as long term potentiation (LTP), ultimately leading to memory impairments and neurodegeneration. Therefore, neuroinflammation may play a critical role in the neurodegenerative processes during the early phases of AD ultimately contributing to the neuropathology that develops in later stages of the disease. The mechanisms outlined in this hypothesis predict that an uncompetitive, moderate-affinity, NMDA channel antagonist could prevent the influx of excessive amounts of calcium ions and attenuate the consequences of the calcium flux dysregulation. In principle, under physiological conditions this would be the role of magnesium ions; however, owing to the modest regional depolarization, magnesium does not provide sufficient blockade of the channel. Memantine, however, can provide sufficient channel blockade that does not interfere negatively with neuronal plasticity (Frankiewicz and Parsons, 1999; Danysz and Parsons, 2003). Indeed, treatment with therapeutically relevant doses of memantine in rats was able to restore *Arc* gene expression to normal levels and, quite surprisingly, reduce the number of activated microglia, an expression of brain inflammation (Fig. 1, right side) (Rosi *et al.*, 2006). These results confirm our current understanding of the role of dynamic changes in *Arc* expression in neuronal plasticity and demonstrate the ability of memantine to reinstate the dynamic balance of cellular processes that were disturbed by chronic brain inflammation.

Chronic neuroinflammation may also be responsible for the selective vulnerability of neurons in AD. Using an animal model of chronic brain inflammation, we have systematically examined, and then selectively inhibited, each step in the cascade (Fig. 2) that leads to excessive stimulation of NMDA receptors and cell death (Wenk and Willard, 1998; Willard *et al.*, 2000; Wenk *et al.*, 2000b). Many of the components of this cascade are shown in this figure.

Following the infusion of lipopolysaccharide, activated microglia can indirectly potentiate glutamate-mediated neurotoxicity via the production of prostaglandins, nitric oxide (Morimoto *et al.*, 2002) and cytokines (Bernardino *et al.*, 2005). The inflammatory process may produce a dysregulation in calcium influx via NMDA receptors that could produce multiple unstable conditions, for example, an elevation in intracellular levels of calcium in a larger than usual proportion of neurons, producing a dramatic increase in the number of neurons with a disruption in neuroplasticity and/or cell death (Soliven and Albert, 1992). Given the critical role of NMDA receptors in this cascade, it is not surprising that chronic neuroinflammation leads to a significant decline in the number of NMDA(R1) receptors, owing to loss of neurons bearing these receptors (Rosi *et al.*, 2004). As predicted by this

Fig. 2



Scheme showing a chain of selected sequential events leading from inflammation to neuronal death. LPS, lipopolysaccharide; NMDA, *N*-methyl-D-aspartate.

hypothesis, the greatest receptor loss occurred in those regions of the hippocampus that also had the greatest concentration of activated microglia. The loss of NMDA receptors in hippocampal regions in response to the presence of chronic neuroinflammation may contribute to the cognitive deficits observed in AD during the earliest phases of the disease (Eikelenboom *et al.*, 1998). As discussed above, an infusion of lipopolysaccharide can lead to excessive excitation of NMDA receptors and a loss of acetylcholine neurons within the basal forebrain; this loss was consistently attenuated by treatment with memantine (Wenk and Willard, 1998; Willard *et al.*, 2000; Wenk *et al.*, 2000a). Clear connections are also found between classical hallmarks of AD such as A β , glutamate and inflammation. It has been shown that aggregated A β produces increase in glutamate secretion by microglia *in vitro* (Gahtan and Overmier, 1999). Moreover, arachidonic acid released by microglia may also enhance NMDA responses and potentiate neurotoxic potential (Miller *et al.*, 1992).

Memantine and 2-amino-5-phosphopentanoic acid as well as soluble tumour necrosis factor- α receptor protected neurons from microglial-conditioned media-dependent death, implicating the excitatory neurotransmitter glutamate and the proinflammatory cytokine tumour necrosis factor- α as effectors of microglial-stimulated death (Floden *et al.*, 2005).

Oxidative stress

Deficits in energy metabolism associated with ageing play an important role in the vulnerability of neurons and in

neurodegenerative diseases, such as AD. A defect in energy production would make neurons that express glutamatergic receptors more vulnerable to elevated or normal levels of endogenous glutamate because decreased levels of intracellular ATP would lead to a partial, and persistent membrane depolarization, the relief of the voltage-dependent Mg^{2+} blockade at NMDA receptors and a prolonged increase in the influx of calcium ions into the cells, a decrease in calcium removal and buffering. In turn, the accumulation of intracellular calcium ions following the activation of NMDA receptors by glutamate would lead to neuronal death. Oxidative stress or impaired buffering of intracellular calcium ions may also result in compromised energy production, possibly leading to impaired function of the membrane ion pumps required for maintenance of the resting potential. In any of these situations, excessive calcium ion influx through NMDA receptors could activate a host of calcium ion-dependent signalling pathways and stimulate nitric oxide production through closely associated neuronal nitric oxide synthase. Nitric oxide can react with a superoxide anion to form peroxynitrite, which disintegrates into extremely toxic hydroxyl-free radicals that can further impair mitochondrial function and energy production. Intracellular calcium may become concentrated within the postsynaptic mitochondria further contributing to the impaired energy production within the region of the NMDA channels (Peng and Greenamyre, 1998; Duchen, 2000).

Mitochondrial dysfunction coupled with activation of glutamatergic receptors could underlie enhanced cholinergic vulnerability associated with ageing and AD. These results suggest that under conditions that lead to a mitochondrial energy deficit, such as that produced by exposure to 3-nitropropionic acid (a naturally occurring toxin that is an irreversible inhibitor of succinate dehydrogenase, complex II), normal synaptic activation of NMDA receptors can lead to the death of the neuron (Wenk *et al.*, 1996). These findings are consistent with the hypothesis that a neurochemical process involving NMDA receptor activation plays a role in neurodegeneration in vulnerable brain regions. Mitochondrial failure may underlie certain progressive neurodegenerative processes that involve the secondary activation of NMDA receptors (Schulz *et al.*, 1996). In addition, mitochondrial dysfunction might have a much greater and earlier impact upon the integrity of cholinergic neurons, in part owing to their dependence upon normal mitochondrial function for the production of acetyl coenzyme A, a precursor to the synthesis of acetylcholine upon normal mitochondrial function. Memantine was also protective in in-vivo models of brain hypoxia and ischaemia (Block and Schwarz, 1996; Chen *et al.*, 1998; Dogan *et al.*, 1999; Ozsuer *et al.*, 2005), conditions associated with increased oxidative stress and enhanced glutamatergic synaptic function (Barnham *et al.*, 2004).

AD is characterized by a forebrain deficiency of acetylcholine (Whitehouse *et al.*, 1981). Although the basis of the vulnerability of cholinergic neurons in AD is not understood, one possibility is that the degeneration of these neurons might be due to excessive stimulation of glutamatergic NMDA and non-NMDA receptors. Throughout the brain, glutamatergic and cholinergic neurons are interrelated in their connectivity and influence on neuroplasticity. Importantly, basal forebrain cholinergic neurons receive a dense glutamatergic projection from the pedunculopontine tegmentum (Mesulam and Mufson, 1984). NMDA receptors are highly concentrated in the region of the basal forebrain that contains a high number of acetylcholine neurons; the loss of these neurons owing to excitotoxicity may contribute to the cognitive deficit observed in AD (Wenk *et al.*, 1994, 2000b; McMillian *et al.*, 1995; Lee *et al.*, 2002). Injection of NMDA receptor agonists such as ibotenic and quinolinic acid, or injection of non-NMDA receptor agonists, such as quisqualic acid or α -2-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid, produced a significant decline in the number of cholinergic neurons. Several studies have shown that the loss of cholinergic neurons caused by injection of NMDA into the rat basal forebrain can be attenuated by pretreatment or cotreatment with therapeutically relevant doses of memantine (Wenk *et al.*, 1994, 1995, 1997; Willard *et al.*, 1999). Memantine also protects against destruction of cholinergic neurons by the mitochondrial toxin 3-nitropropionic acid, which may act by undermining the production of energy, leading to a reduction in the resting membrane potential and the loss of the magnesium ion blockade at the NMDA channel (Wenk *et al.*, 1996) according to the excitotoxic mechanism described above. Acetylcholine neurons rescued from toxicity will then be available to respond to acetylcholinesterase inhibitor therapies. Similar neuroprotection against 3-NP neurotoxicity was observed in the rat striatum (Schulz *et al.*, 1996). In organotypic hippocampal slices *in vitro*, memantine was protective with an IC_{50} of around 1–2 μ mol/l against semichronic excitotoxicity (4–20 days) produced by 3-NP (Karanian *et al.*, 2006).

Moreover, if memantine can prevent abnormal glutamate neurotransmission, it may provide neuroprotection in both the early stages of many different neurodegenerative diseases when toxicity is generated and later when symptoms are apparent. Given the consequences of chronic neuroinflammation upon the overactivation of NMDA receptors, the increased entry of calcium ions, and the subsequent loss of acetylcholine neurons expressing these receptors in vulnerable brain regions, long-term prophylactic treatment of AD patients with memantine should significantly lessen the early consequences of the brain inflammation, attenuate the loss of neuroplasticity and improve learning and memory abilities.

Beta-amyloid

AD is characterized by progressive deterioration of cognition and memory, and disturbed emotional reactivity caused by dysfunction and degeneration of neurons in the limbic system and cerebral cortex. Affected brain areas typically contain extracellular neuritic plaques comprising fibrillar A β deposits, and intracellular neurofibrillary tangles comprising paired helical filaments of hyperphosphorylated-tau. The deposition of A β is probably a key element leading to the neuronal loss seen in the AD brain. The 'amyloid hypothesis' of AD posits that the gradual accumulation of A β in the interstitial fluid of the brain oligomerizes, providing a focus for the subsequent deposition of other proteins. Inflammatory proteins released by the activated glia may promote the transformation of diffuse β -amyloid deposits into a filamentous and possibly more neurotoxic form (Schubert *et al.*, 1998). This accumulation of toxic fibrillar A β injures neurites within the plaques and in the surrounding neuropil. Such injury disrupts both neuronal function and homeostasis, and eventually causes neuronal death. Although the manner in which A β damages neurons is not completely understood, both oxidative stress and disruption of neuronal Ca²⁺ homeostasis, resulting in excitotoxicity, have been implicated (Cowburn *et al.*, 1997). A β can stimulate microglia to secrete cytokines and reactive oxygen species (Meda *et al.*, 1999). A β also induces oxidative stress and perturbs neuronal ion homeostasis by promoting membrane lipid peroxidation, which can impair the function of membrane-bound ion, glucose and amino acid (including glutamate) transport proteins. In addition to producing oxidative stress and affecting Ca²⁺ homeostasis, A β may increase the vulnerability of neurons to glutamate, leading to glutamate excitotoxicity and the opportunity for memantine to reduce this vulnerability. Independent from neuronal loss, A β may also contribute to disrupted neuronal plasticity of remaining neurons in concert with glutamate as shown for LTP in hippocampal slices (Nakagami and Oda, 2002).

Several factors potentially contribute to the inability of neurons to maintain normal resting membrane potential in the AD brain. For example, A β can chronically depolarize neurons through its action on the metabotropic glutamate receptor 1 (Blanchard *et al.*, 2002). Such A β -induced membrane depolarization would be expected to partially relieve voltage-dependent Mg²⁺ block of NMDA receptors. Under these conditions, subsequent activation of NMDA receptors by ordinary glutamatergic synaptic activity could permit a continuous entry of calcium ion into neurons, theoretically overwhelming the endogenous mechanisms that regulate calcium homeostasis. Therefore, neurons that express NMDA receptors would become selectively vulnerable to normal glutamatergic stimulation. This is similar to the situation

described above owing to the presence of chronic brain inflammation. A β may also inhibit glial glutamate uptake (Harris *et al.*, 1996) and directly enhance NMDA receptor function (Wu *et al.*, 1995).

A β can interact with NMDA receptors and enhance NMDA receptor-mediated excitotoxicity. For example, radioligand-binding experiments in rat cortical membranes suggest that A β selectively binds to the glutamate and glycine binding sites of the NMDA receptor, and not to non-NMDA glutamate receptor subtypes (Cowburn *et al.*, 1997). This binding may be functionally important, inasmuch as application of A β to rat hippocampus slices can enhance NMDA receptor-mediated postsynaptic neuronal responses (Wu *et al.*, 1995). Mature cultured murine cortical neurons and fetal human cerebral cortical cell cultures exposed to A β were more susceptible to excitotoxic injury by glutamate or NMDA, as compared with neurons that were not exposed to A β (Kim and Ko, 1998). Given the role of the NMDA channel in the vulnerability of neurons, it was not surprising that a chronic infusion of memantine reduced local neuronal cell loss produced by the intrahippocampal injection of A β (Miguel-Hidalgo *et al.*, 2002). Recently, the same group reported inhibition of apoptosis induced by A β infused in the hippocampus by memantine. Neuroprotective effects of memantine against A β toxicity have also been shown in functional terms. Thus, Yamada and co-workers (2005) recently showed that infusion of memantine prevents the development of delayed nonmatching to sample lever pressing task produced by infusion of A β in rats. Interestingly, *in vitro*, brief exposure of cultured cortical neurons to memantine, which would produce only a transient block of NMDA receptors, inhibited the toxicity of A β for up to 48 h (Tremblay *et al.*, 2000). The relevance of this brief effect of the memantine with regard to chronic therapy in AD remains to be investigated.

In a transgenic murine model of AD, accelerated amyloid deposition in hippocampus and cortex is associated with dystrophic neurites and reactive astrocytes (Price *et al.*, 1998). In transgenic mice (APP23), memantine treatment prevented a decrease in the performance as seen with time in vehicle-treated animals and this disease-modifying-like effect was observed 3 weeks after treatment termination (Van Dam and De Deyn, 2006). Transgenic mice (APPswe) treated chronically with memantine also show lower levels of membrane-bound amyloid precursor protein (APP) (Unger *et al.*, 2005). Recent studies using cultured human neuroblastoma (SK-N-SH) cells have demonstrated memantine may decrease amyloid processing (Lahiri *et al.*, 2003). It remains to be determined whether memantine can produce a similar disease-modifying effect in the AD brain.

Tau

The evidence discussed above indicates a clear negative effect of A β on the function of NMDA receptors. In addition, NMDA receptor function shows positive feedback interactions with the expression and functional state of tau. Tau is a microtubule-associated protein that promotes microtubule polymerization and stabilization. Hyperphosphorylated tau accumulates in paired helical neurofilaments to form neurofibrillary tangles in the brains of patients with AD. It is noteworthy, according to some groups, that phosphorylated tau correlated better with cognitive decline than A β accumulation (Braak and Braak, 1995). Glutamatergic cortical pyramidal neurons, which are affected early in the disease, are subject to tangle formation (Francis *et al.*, 1999). A potential link between glutamate-induced excitotoxicity and tau was first demonstrated by studies using cultured rat hippocampal neurons; glutamate-induced neurodegeneration was associated with immunostaining that was specific for the presence of neurofibrillary tangles (Mattson, 1990).

In rat brain primary cultures, a tau antisense oligonucleotide decreased neuronal sensitivity to excitotoxic injury (Pizzi *et al.*, 1993) [but see Lesort *et al.* (1997) for contrasting data]. Acute or chronic NMDA-induced excitotoxicity in neuronal cultures can also significantly enhance tau production (Sindou *et al.*, 1992; Pizzi *et al.*, 1995) and selectively increases phosphorylated tau (Couratier *et al.*, 1996). Given the potentially significant role of neurofibrillary tangle formation in the clinical progression of AD dementia (Bierer *et al.*, 1995) and the fact that glutamate increases tau phosphorylation (Sindou *et al.*, 1994), which can be prevented by NMDA receptor antagonists (Couratier *et al.*, 1996), it is very likely that NMDA receptor-dependent influence upon tau phosphorylation promotes the evolution of AD pathology and dementia. Therefore, current evidence supports a critical role for tau in the neural processes associated with excitotoxic neurodegeneration. The abnormal hyperphosphorylation of tau may be related to the impaired activity of protein phosphatase (PP)-2A; memantine exposure restored reduced phosphorylation of tau (ser262) and neurotoxicity produced by okadaic acid in a rat hippocampal slice preparation (Li *et al.*, 2004). It is not clear whether this effect is solely due to NMDA antagonism because competitive antagonists and antagonists acting at the glycine site of the NMDA receptor were ineffective, at least at the concentrations used (Li *et al.*, 2004). Recently, further insights into the possible mechanism of action became available, namely, it was reported that memantine caused an increase in the phosphorylation of GSK-3 which inhibits GSK-3 function and could thereby reduce phosphorylation of presenilin 1 and 2 and tau (De Sarno *et al.*, 2006). This could potentially contribute to neuroprotective effects in AD. It has been recently shown that, after chronic treatment of AD patients with memantine for 12 months, cerebrospinal fluid levels of

phosphorylated tau decrease whereas unphosphorylated tau and A β remain unchanged (Dr Malin Degerman Gunnarsson, personal communication). Taken together, these findings suggest that memantine might be useful for the treatment of AD and related tauopathies.

Therapeutic relevance

Overall, the above presented evidence indicates that memantine and other NMDA antagonists have potential for powerful protective activity against neurodegeneration involving glutamate excitotoxicity, under diverse experimental conditions. To the extent that similar mechanisms contribute to cell death in AD and age-related neurodegenerative diseases, memantine could theoretically slow their progression as well. In turn, glutamate antagonists should be investigated for their potential neuroprotective actions in preclinical and clinical trials. In the case of AD, we are dealing with a disease in which multiple factors, such as glutamatergic dysfunction, inflammation, oxidative stress, A β and tau, not only contribute to the cell death but also interact with each other, leading to exaggerated pathology through positive feedback. The results presented above indicate that glutamate and NMDA receptors, in particular, play a central role here, as at least a part of the neurodegeneration induced by these conditions is clearly mediated via NMDA receptors – as evidenced by the protective effects of memantine in numerous models.

Recently, Olney and colleagues (Creeley *et al.*, 2006) have argued that it is impossible to block NMDA receptors sufficiently to provide neuroprotection without side effects such as memory impairment. This statement, however, ignores the fact that magnesium is an NMDA receptor antagonist and it is present in the brain in concentrations blocking NMDA receptors. Magnesium is neuroprotective because its removal may produce neurodegeneration, but at the same concentrations, magnesium does not produce side effects, and in particular, does not impair learning; in contrast, removal of magnesium does impair neuronal plasticity (Coan *et al.*, 1989). Moreover, the kainate neurodegeneration model used by Creeley *et al.* (2006) to assess neuroprotection is an acute model and not relevant for AD. Another aspect is that higher, toxic plasma levels of memantine might be expected in this study, owing to the use of female rats and older animals (6–8 months) than those typically used for pharmacological studies. Older rats show higher plasma levels of memantine than young animals: for example, infusion of memantine 24 mg/kg/day leads to plasma levels of around 1.4 μ mol/l in 2–3-month-old rats and 4 μ mol/l at 24 months (Danysz, unpublished). Similarly, after infusion or acute injection of memantine, serum levels up to two times higher are seen in females than in male rats (Zajackowski *et al.*, 2000). Therefore, the treatment regime used by Creeley *et al.* (2006) may well

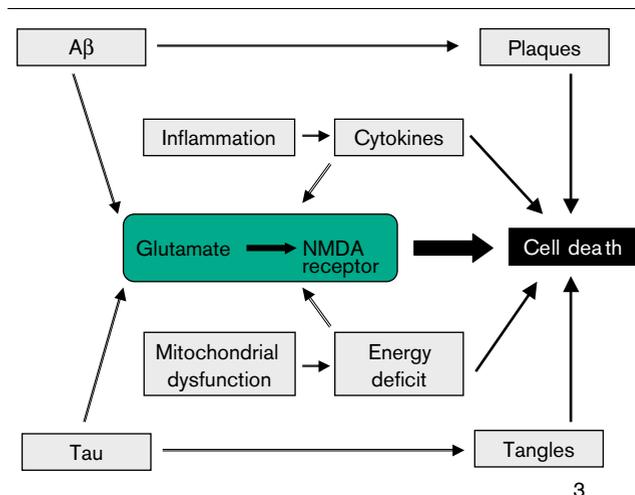
have led to very high levels of memantine, despite the fact that the doses *per se* appear to be quite low. The study by Creeley *et al.* (2006) also questioned whether memantine has a higher therapeutic safety index (TI) than other NMDA antagonists such as MK-801 or PCP. This was, however, based on retrospective reference to the authors' earlier data, while ignoring other studies that made direct comparisons. For example, the TI for neuroprotection vs. ataxia, myorelaxation and stereotypy was 1.18–1.68 for (+)MK-801 vs. 5.59–8.58 for memantine (Wenk *et al.*, 1995); the TI for neuroprotective vs. memory impairing doses was 1.3 for (+)MK-801 vs. 7.1 for memantine (Miszta and Danysz, 1995; Wenk *et al.*, 1995); and in mice, the comparison of ataxia as a measure of side effects vs. inhibition of maximal electro-shock convulsions as a measure of NMDA antagonism resulted in a TI of 1 for (+)MK-801 vs. 2.5–3 for memantine (Parsons *et al.*, 1995).

A major problem in identifying the neuroprotective actions of a drug is related to the long treatment durations (1–3 years) that are required to demonstrate true protection from the neurodegenerative processes that are thought to underlie AD or other age-associated diseases. Drug toxicity and side effect profiles also become more important in the elderly population, further increasing problems with dropouts. Often discrepancies exist between the results of preclinical studies and clinical trials that underlie some of the disappointing outcomes. In clinical trials, neuroprotective efficacy will need to be measured by improved behavioural and neurological function. Such trials aimed at showing neuroprotection require both placebo control groups and a relatively long washout period from the drug, to ensure that testing is performed when drug is not present in the brain. Clear ethical concerns exist about such clinical trial designs. Also, some animal models may be poor predictors of clinical trial results. Important and often unrecognized differences are found in the composition and response of the brain of rodents and humans. For example, more than 90% of brain tissue in rodents is composed of grey matter, while in humans grey matter accounts for only about 50% of the brain wet weight. Therefore, in animal model studies with neuroprotective agents, various tests of functional impairment may not reflect or predict those observed in clinical trials. Numerous functional measures have been developed for animal studies, including the T-maze tests, Morris water pool and radial arm maze performance; these tests are often chosen with regard to the expertise of the investigator and not necessarily because the tests represent important aspects of functional outcome in animals. In many preclinical studies, neuroprotective agents are typically given before or immediately after the onset of neural injury, such as some described above. As a result of the nature of the degenerative processes occurring in the AD brain, this type of treatment of

animals is simply inappropriate as a means of predicting meaningful neuroprotection in humans. Obviously, any drug that has neuroprotective properties will require long follow-up periods to allow beneficial effects to be clearly documented. Neuroprotective trials will need to utilize drugs that target different aspects of the known neurodegenerative cascade. Therefore, best results may be achieved by using their combination, particularly if the intent is to demonstrate synergistic effects over time. Combination drug therapies may require that the dose of each drug be reduced in order to limit drug toxicity; unfortunately, combination therapy will likely add further to the complexity of the trial design. Combination therapy could consist of agents targeting each of the above discussed mechanisms such as inflammation, oxidative stress, A β and tau; however, as shown above, neurodegeneration produced by each of these mechanisms involves NMDA receptors as exemplified by neuroprotective effects of memantine. Thus, an alternative approach is inhibition of pathomechanism by a single agent such as memantine (Fig. 3).

So far, there have been few attempts to show neuroprotective activity in AD. Investigators have found a significant association between exposure to NSAIDs for more than 2 years and AD risk reduction (Breitner *et al.*, 1994). Recent studies suggest that, although anti-inflammatory agents do not appear to slow down the progression of dementia, they may have a preventive influence on the development of AD pathology (Breitner *et al.*, 1994; Wenk *et al.*, 2000a; Marchetti and Abbracchio, 2005). Multiple clinical trials have, however, elicited mixed (Rogers *et al.*, 1993; Andersen *et al.*, 1995; in t' Veld

Fig. 3



Scheme showing the relationship between inflammation, A β , tau, oxidative stress and glutamatergic system contributions to neurodegeneration. Black filled arrows indicate processes leading directly to neuronal loss. A β , amyloid- β ; NMDA, N-methyl-D-aspartate.

et al., 2001), albeit mostly negative, results (McGeer *et al.*, 1990). We predict that NMDA receptor antagonism by memantine should provide the first effective neuroprotective therapy. Indeed, a small clinical study in Huntington's patients already suggests some indication for neuroprotective activity in the clinical setting (Beister *et al.*, 2004).

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