Potential role of N-methyl-d-aspartate receptors as executors of neurodegeneration resulting from diverse insults: focus on memantine
Gary L. Wenka, Chris G. Parsonsb and Wojciech Danyszb

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. 

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Keywords: Alzheimer’s disease, neurodegeneration, neuroprotection, N-methyl-D-aspartate receptor antagonist

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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 Ca2+ and contribute to fast synaptic transmission. In contrast, NMDA receptors are characterized by high permeability to Ca2+ ions, voltage-dependent blockade by Mg2+ 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 Mg2+ 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, Mg2+ is no longer strongly attracted into the pore of the channel and dissociates. Under such depolarized conditions, NMDA receptors activated by synthetically released glutamate allow the influx of Na+ and, in particular Ca2+, 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 Ca2+ overload. Namely, upon mild depolarization, magnesium block is removed and NMDA channels remain open (permeable to Ca2+ 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.

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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 (Aβ) 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-o-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; Mildner, 1982a, b; Schneider et al., 1984; Mundinger and Milios, 1985). The initial effects of memantine in animal studies resembled dopaminomimetics (Maj, 1982; Wese- mann et al., 1983); however, these effects were only observed at plasma levels that were much higher than those achieved in patients with AD (Danyasz 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 steadystate (chronic treatment for several weeks), plasma levels of memantine are in a range of 0.4–1 μmol/l (Kornhuber and Quack, 1995; Danyasz et al., 1997; Periclo 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) (Danyysz unpublished; Danyasz 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 μmol/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 μmol/l, then any receptor that expresses an affinity of low μmol/l or lower should be considered as a potential target. Given this assumption, there are only four plausible targets: the NMDA receptor channel, 5-HT\(_3\) receptors and the nicotinic receptors of α7 or α4β2 type (Buisson and Bertrand, 1998; Maskell et al., 2003; Rammes et al., 2004; Aracava et al., 2005). Antagonism of the 5-HT\(_3\) 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 odansetron or granisetron (Lehmann and Karrberg, 1996; Merz unpublished data) indicating a lack of 5-HT\(_3\) blockade after behavioural active doses. Memantine can inhibit α4β2 responses with an IC\(_{50} = 6.6\) μmol/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 α4β2 receptors (Mansbach et al., 2000), at doses that also affected saline responding, indicating lack of selectivity. The reported potency of memantine at α7 receptors varies considerably from 0.33–1.68 μmol/l seen in rat receptors (Aracava et al., 2005) to 5 μmol/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)

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<td>Memantine prolongs survival in transgenic model of ALS (SOD mice)</td>
<td>Wang and Zhang, 2005</td>
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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.
memantine is more effective surrogate for magnesium ions as it serves as a more effective blocker of NMDA receptors than magnesium ions, for example owing to partial voltage-dependence, such as dizocilpine [(+)-5-methyl-10,11-dihydro-5H-dibenzo[c,e]cyclohepten-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). Antagonists that have 'too high' affinity for the channel or 'too little' voltage dependence, such as dizocilpine [(+)-5-methyl-10,11-dihydro-5H-dibenzo[c,e]cyclohepten-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.

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 blocks 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 Mg2+, albeit with greater affinity. Additionally, this hypothesis (Lipton, 2006) does not provide an explanation for symptomatic 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 pathophysiological 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 anti-inflammatory 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 Aβ (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 (Katsuura 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 involved in memory consolidation, leading to cognitive impairment.

**Fig. 1**

Immunofluorescence staining for the Arc protein (red) and activated microglia (blue) within the Dendate 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 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 Aβ, glutamate and inflammation. It has been shown that aggregated Aβ 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-phosphopetanoic 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

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**Fig. 2**

Scheme showing a chain of selected sequential events leading from inflammation to neuronal death. LPS, lipopolysaccharide; NMDA, N-methyl-D-aspartate.
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 μmol/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 Aβ deposits, and intracellular neurofibrillary tangles comprising paired helical filaments of hyperphosphorylated-tau. The deposition of Aβ 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 Aβ 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 Aβ 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 Aβ 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). Aβ can stimulate microglia to secrete cytokines and reactive oxygen species (Meda et al., 1999). Aβ 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, Aβ 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, Aβ may also contribute to disrupted neuronal plasticity of remaining neurons in concert with glutamate 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, Aβ can chronically depolarize neurons through its action on the metabotropic glutamate receptor 1 (Blanchard et al., 2002). Such Aβ-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. Aβ may also inhibit glial glutamate uptake (Harris et al., 1996) and directly enhance NMDA receptor function (Wu et al., 1995).

Aβ can interact with NMDA receptors and enhance NMDA receptor-mediated excitotoxicity. For example, radioligand-binding experiments in rat cortical membranes suggest that Aβ 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 Aβ 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 Aβ were more susceptible to excitotoxic injury by glutamate or NMDA, as compared with neurons that were not exposed to Aβ (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 Aβ (Miguel-Hidalgo et al., 2002). Recently, the same group reported inhibition of apoptosis induced by Aβ infused in the hippocampus by memantine. Neuroprotective effects of memantine against Aβ 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 Aβ 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 Aβ 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 (APPsw) treated chronically with memantine also show lower levels of membrane-bound amyloid precursor protein (APP) (Unget 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 Aβ 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 Aβ accumulation (Braak and Braak, 1995). Glutamatergic cortical pyramidal neurons, which are affected early in the disease, are subject to tangle formation (Francis et al., 1992). 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 Aβ 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 agerelated 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, Aβ 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 (Goan 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 μmol/l in 2–3-month-old rats and 4 μmol/l at 24 months (Daniy, unpublished). Similarly, after infusion or acute injection of memantine, serum levels up to two times higher are seen in females than in male rats (Zajaczkowski 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 neuroprotection vs. memory impairing doses was 1.3 for (+)MK-801 vs. 7.1 for memantine (Misztal and Danyasz, 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, Aβ 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](image-url)

**Scheme showing the relationship between inflammation, Aβ, tau, oxidative stress and glutamatergic system contributions to neurodegeneration.** Black filled arrows indicate processes leading directly to neuronal loss. Aβ, 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 neuropro- tective 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).

References


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