callosal lesions in the above-mentioned 3 patients thus suggests that the callosal fibers for writing are concentrated in the posterior end of the main callosum, while those for praxis cross in the more rostral part of the posterior half of this trunk. Clinical studies on stroke patients [9] and patients with surgical section of the corpus callosum [10] support this view. In addition, since the left-sided apraxia observed in the patient described by Kawamura and associates [7] was resolved at 4 months after the ictus, and that in our patient resolved at 1 month after the ictus, the callosal pathways subserving praxis not only may be distributed densely in the posterior half of the callosal trunk but also may be sparsely present in the anterior callosum. Residual intact cross-communications through the anterior part of the corpus callosum might play an important role in the recovery of patients from callosal apraxia.

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Delayed Administration of Memantine Prevents *N*-Methyl-D-Aspartate Receptor–mediated Neurotoxicity

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Increasing evidence supports the hypothesis that escalating levels of excitatory amino acids (EAAs) are responsible for neuronal cell death in a variety of acute neurological conditions including hypoxia/ischemia, trauma, seizures, and hypoglycemia. EAAs may also contribute to several chronic neurodegenerative diseases including Huntington's disease, parkinsonism, and acquired immunodeficiency syndrome dementia. A predominant form of neurotoxicity appears to be mediated by excessive activation of the *N*-methyl-D-aspartate subtype of glutamate receptor. This laboratory recently reported that memantine, an antiparkinsonian drug, is a potent *N*-methyl-D-aspartate antagonist capable of preventing the death of central neurons both in vitro and in vivo when given coincident to an EAA insult. In the present study, we found that 12 μM memantine prevented the death of neonatal rat retinal ganglion cells in primary culture when administered up to 4 hours after the initiation of *N*-methyl-D-aspartate receptor–mediated neurotoxicity.

Pellegrini JW, Lipton SA. Delayed administration of memantine prevents *N*-methyl-D-aspartate receptor–mediated neurotoxicity. Ann Neurol 1993;33:403–407

Glutamate has been implicated as a significant factor in the neurotoxicity associated with hypoxic/ischemic encephalopathy, seizures, trauma, and several degenerative neurological disorders such as Huntington's disease, parkinsonism, and acquired immunodeficiency syndrome dementia [1–3]. In many central neurons, the predominant form of this toxicity appears to be mediated by overstimulation of the *N*-methyl-D-

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aspartate (NMDA) subtype of glutamate receptor with subsequent influx of excessive Ca^{2+}, possibly leading to a series of events associated with a lethal outcome. Memantine (1-amino-3,5-dimethyladamantane hydrochloride) is a drug used in the treatment of Parkinson's disease in Europe, and is an analogue of amantadine, a well-known antiviral and antiparkinsonian agent that has been used clinically for >20 years [4]. Recently, memantine, and to a lesser degree, amantadine and other adamantane derivatives, have been shown to produce reversible open-channel block of NMDA receptor–operated ion channels [5, 6]. Moreover, unlike MK-801 {dizocilpine; (+)-5-methyl-10,11-dihydro-5*H*-dibenzo [*a,d*] cyclohapten-5,10-imine hydrogen maleate} and most other organic NMDA antagonists, memantine and its derivatives are clinically tolerated drugs in humans with relatively few side effects. In fact, concentrations of memantine known to be tolerated by patients taking the drug for parkinsonism can prevent NMDA receptor–mediated neurotoxicity in animal models both in vitro and in vivo when administered coincident with an excitatory amino acid (EAA) insult [6]. However, of significant clinical importance is the capacity of such a compound to prevent neuronal cell death when administered at a time interval after the initial insult. Therefore, in this study we investigated the effect of delayed administration of memantine on the survival of primary cultures of neonatal rat retinal ganglion cells after a potentially toxic exposure to glutamate.

Methods

Neuronal Labeling, Dissociation, and Culture Techniques

We used techniques developed in this laboratory that have been detailed elsewhere [7, 8]. In brief, retinal ganglion cells of 4 to 6-day-old Long–Evans rats were labeled in situ with the fluorescent dye granular blue by injection of the dye into the superior colliculus followed by its retrograde transport. Two to 6 days later, the animals were killed by decapitation. After enucleation, the retinas were dissociated by mild treatment with the enzyme papain. The retinal cells were then plated onto glass coverslips coated with poly-L-lysine in 35-mm tissue culture dishes containing 2 ml of growth medium with additives as described below, incubated for ~20 hours at 36°C in a humidified atmosphere of 5% CO_2/95% air, and then assessed for viability. Short-term cultures were used to minimize morphological and biochemical changes that neurons might undergo when exposed to artificial culture conditions for prolonged periods of time. The growth medium contained Eagle's minimum essential medium supplemented with 16 mM glucose, 2 mM glutamine, 0.7% (wt/vol) methylcellulose, 5% (vol/vol) rat serum, and 1 μg/ml of gentamicin. In the neuronal survival experiments, we monitored the effect of memantine on the viability of acutely isolated retinal ganglion cells that were cultured in control medium (1.8 mM Ca^{2+}, 0.8 mM $MgCl_2$) or in high (10 mM) Ca^{2+}, low (50 μM) Mg^{2+}; the latter medium is known to enhance NMDA receptor–mediated toxicity in this preparation due to an endogenous glutamate level of ~25 μM [7–9]. This form of excitotoxicity can be attenuated with the NMDA-specific antagonists D-2-amino-5-phosphonovalerate (APV; 200 μM) or MK-801 (2–20 μM) [8]. Furthermore, previous patch–clamp studies in this laboratory have shown that NMDA-evoked current responses are present in cultured retinal ganglion cells of this age [10, 11], consistent with the fact that the major form of excitotoxicity in this preparation is mediated by overstimulation of NMDA receptors [12]. In another set of experiments, to ensure the synchronous initiation of excitotoxicity, 200 μM exogenous NMDA was added to the culture medium at the time of plating.

Memantine (12 μM) was added to the growth medium at time intervals of 0, 1, 4, and 7 hours after plating the retinal cells. This concentration of memantine was chosen for two reasons. First, in previous experiments with dose–response curves, we had found that this concentration of memantine was maximally effective in protecting rodent neurons from NMDA receptor–mediated toxicity when added at the time of the excitotoxic insult [6]. Second, approximately the same concentration is known to be present in the human brain of patients taking memantine as an antiparkinsonian agent [13]. After overnight incubation, neuronal survival was assessed using a protocol that we have described in detail previously [7, 8]. Surviving cells were scored by their ability to take up and cleave fluorescein diacetate to fluorescein, and retinal ganglion cells were specifically identified by the presence of the retrogradely transported dye granular blue. Experiments were performed in triplicate and replicated on three separate occasions.

Results

Our results substantiate an earlier finding in the retinal preparation [7, 8], which showed that an endogenous glutamate-like agonist produces neurotoxicity in the presence of elevated extracellular calcium concentrations (Fig 1, compare columns 1 and 2). This form of neuronal cell death is mediated by activation of the NMDA receptor because the NMDA receptor-specific antagonist APV (200 μM) completely attenuates toxicity engendered by the endogenous substance [7]. Preliminary experiments indicate that the endogenous toxin is glutamate itself [9]. It is relevant that the endogenous toxic factor in this culture system is glutamate because the same EAA is thought to underlie at least in part the neuronal damage observed in stroke and other neurological disorders [1–3].

Treatment of retinal cultures with memantine (12 μM) up to 4 hours after the initiation of NMDA receptor–mediated neurotoxicity had significant neuroprotective effects on the retinal ganglion cells (see Fig 1, columns 3–5). The potency of this neuronal salvage effect appears to decrease with time. When memantine was added at time zero (i.e., coincident with the initiation of NMDA receptor–mediated neurotoxicity), cell survival was actually above control levels (see Fig 1,

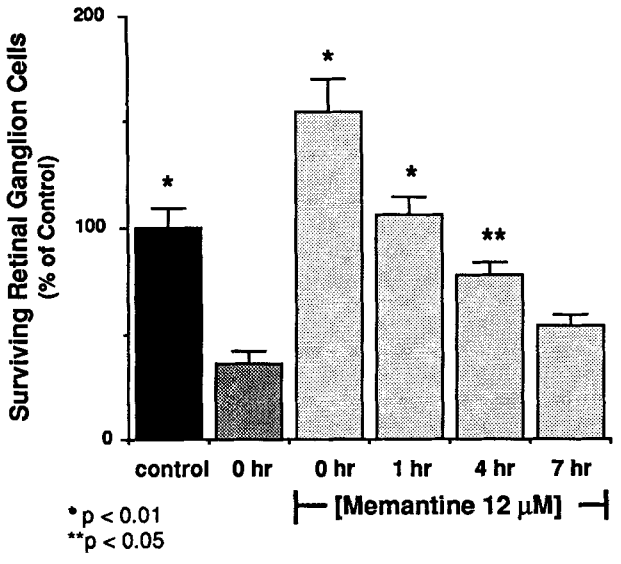

Fig 1. Delayed administration of memantine protects rat retinal ganglion cell neurons from N-*methyl-*D*-aspartate (NMDA) receptor–mediated toxicity due to endogenous glutamate agonist. Control dishes contained normal $CaCl_2$ (1.8 mM) and $MgCl_2$ (0.8 mM), whereas the other categories were exposed to supraphysiological $CaCl_2$ (10 mM) and low $MgCl_2$ (50 μM) to facilitate NMDA receptor–mediated neurotoxicity in this preparation. Viability of retinal ganglion cells was assayed after overnight exposure to control conditions (column 1), or after incubation in high Ca^{2+}/low Mg^{2+} with or without memantine as follows: no added memantine (only saline diluent added at the time of cell plating; column 2), memantine (12 μM) added at the time of cell plating (column 3), or memantine added 1, 4, or 7 hours later (columns 4, 5, and 6, respectively). Under these conditions, memantine enhanced retinal ganglion cell survival when administered up to and including the 4-hour mark. The data represent three separate experiments, each conducted in triplicate on identical culture dishes and normalized to their respective controls; typically there were ~150 viable retinal ganglion cells in each control culture dish. The values shown are the mean + SEM. Statistical tests were performed with a one-way analysis of variance followed by a post hoc Fisher protected least significant difference test with Bonferroni correction for multiple comparisons. *p < 0.01; **p < 0.05, significance of increase in viability compared with the level of NMDA receptor–mediated neurotoxicity observed in the absence of memantine (column 2).*

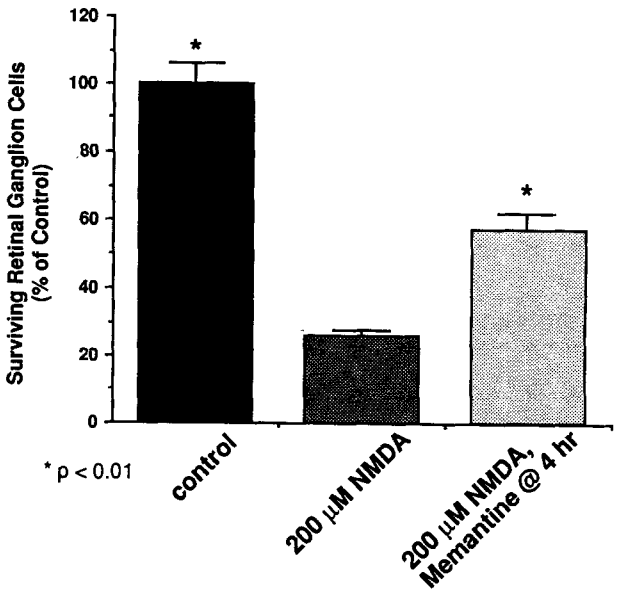

Fig 2. Delayed administration of memantine protects rat retinal ganglion cell neurons from excitotoxicity due to exogenous N-*methyl-*D*-aspartate (NMDA). Control dishes contained normal $CaCl_2$ (1.8 mM) and $MgCl_2$ (0.8 mM). The other categories were exposed to 200 μM NMDA from the time of cell plating, in supraphysiological $CaCl_2$ (10 mM) and low $MgCl_2$ (50 μM) to facilitate NMDA receptor–mediated neurotoxicity. (Hence, the media were the same as in Figure 1, except 200 μM NMDA was added to all but the control cultures.) Under these conditions, the addition of 12 μM memantine ameliorated NMDA receptor–mediated neurotoxicity when added 4 hours after the initiation of injury (compare columns 2 and 3). *p < 0.01, significance of increase in viability compared with the level of NMDA receptor–mediated neurotoxicity observed in the absence of memantine (column 2).*

compare columns 1 and 3). This finding is consistent with previously reported results in this preparation [7–9] showing that some degree of NMDA receptor–mediated neuronal cell death occurs even in a normophysiological calcium environment (1.8 mM Ca^{2+}) in the presence of the endogenous glutamate. The cells treated with memantine at 1 hour survived at "control" levels (compare columns 1 and 4), whereas those treated at 4 hours survived at numbers approximating 80% of control. In contrast, the addition of memantine 7 hours after the initiation of the insult did not significantly protect retinal ganglion cells (see Fig 1, column 6).

In another set of experiments, we added an exogenous EAA (200 μM NMDA) to the culture medium at the time of cell plating to ensure that excitotoxicity was initiated synchronously (at time = 0 hr). Under these conditions, the administration of 12 μM memantine at 4 hours after plating still resulted in significant neuronal salvage (Fig 2).

Discussion

It is currently thought that acute insults to the central nervous system (CNS), including stroke and trauma, are mediated by overstimulation of NMDA receptors, resulting in delayed neurotoxicity [1, 2]. Therefore, any future effort to combat this form of excitotoxic damage in the brain will have to use drugs that can prevent neuronal injury when administered after the initial insult, i.e., after the patient presents with a clinical deficit. Thus, many laboratories are currently

searching for clinically tolerated NMDA antagonists that can be used at some time interval after an acute neurological event [8, 14, 15]. In the present study, we describe in vitro experiments that suggest that the drug memantine may display many of the characteristics necessary to fit these clinical requirements, at least for the salvage of retinal ganglion cell neurons. Memantine, a more potent congener of amantadine, has been used clinically for many years in Europe. Most importantly, memantine concentrations similar to those used in the current experiments can be attained in humans in the absence of significant side effects [13, 16].

In the present article, we report that delayed treatment with memantine can prevent NMDA receptor–mediated neurotoxicity in a model system containing mammalian central neurons. Explaining these results, we recently reported that memantine inhibited NMDA-evoked ionic currents by a mechanism of open-channel block [6]. Our previous data further indicated that memantine and, to a lesser extent, amantadine can prevent NMDA receptor–mediated Ca^{2+} influx and subsequent neuronal cell death when administered at the time of the EAA insult [6]. The present study reveals that delayed administration of memantine, up to 4 hours after the initial insult, can also significantly ameliorate NMDA receptor–mediated neurotoxicity in vitro of rat retinal ganglion cells.

Moreover, in patch–clamp experiments [6], at a given concentration of memantine, the effects of high concentrations of NMDA were blocked to a relatively greater degree than low concentrations of the agonist. This mechanism (termed uncompetitive inhibition) offers both theoretical and practical advantages; the normal consequences of a lower degree of activation of NMDA receptors should be at least partially spared (e.g., the processes involved in eliciting long-term potentiation, thought to represent a cellular correlate of learning and memory). In contrast, at other sites in the brain, this mode of action would result in concomitant blockade of the lethal effects of escalating levels of glutamate, as observed after a stroke or trauma to the CNS. For example, in the penumbra of a stroke or in the face of ongoing ischemic damage, memantine would produce a greater degree of blockade as the concentration of glutamate increases due to its release from dying neurons.

Heretofore, no drug in common clinical usage for other ailments was known to be tolerated at dosages that might be effectively administered to patients facing acute neurological insults, such as stroke and trauma. Given our preliminary in vitro data, we are encouraged that delayed treatment with memantine and its analogues should undergo further testing, first in a variety of neuronal cell types such as cortical and cerebellar neurons. If also useful for these types of neurons, then perhaps memantine can be evaluated in animal models for possible therapeutic application in a wide range of neurological problems that appear to be mediated at least in part by overstimulation of NMDA receptors, including hypoxic/ischemic brain and retinal injury, head and spinal cord trauma, and possibly several neurodegenerative disorders. The fact that drugs in the adamantane class are already used clinically for other indications may expedite their testing and eventual application in humans.

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Successful Outcome of Progressive Multifocal Leukoencephalopathy with Cytarabine and Interferon

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The prognosis of patients with progressive multifocal leukoencephalopathy is poor, with few patients showing remission or surviving. We describe a 37-year-old man who developed progressive multifocal leukoencephalopathy in association with sarcoidosis. Despite treatment with cytarabine and acyclovir, he continued to deteriorate. Shortly following the addition of interferon alfa, he made a dramatic improvement, regaining full functional independence. The use of interferon alfa in addition to cytarabine in such patients offers a new therapeutic approach worthy of further trial.

Steiger MJ, Tarnesby G, Gabe S, McLaughlin J, Schapira AHV. Successful outcome of progressive multifocal leukoencephalopathy with cytarabine and interferon. Ann Neurol 1993;33:407–411

Progressive multifocal leukoencephalopathy (PML) is typically associated in patients with an impairment in cell-mediated immunity. The first description of the disease by Astrom and colleagues [1] in a review of the literature suggested additional associations, with similar histopathological changes in cerebral white matter occurring in a patient with sarcoidosis. This adult patient had been described 3 years earlier by Christensen and Fog as having Schilder's disease [2]. Over the last 33 years, an association of PML with sarcoidosis has occasionally been described [3, 4], but with no clear relationship between the onset of neurological symptoms and either the severity or the duration of the sarcoidosis.

Although spontaneous partial recovery and prolonged survival are recognized in PML [5], with one autopsy-confirmed case of 33 years' duration [6], the disease is almost invariably relentlessly progressive, with a life expectancy of 3 to 18 months [3, 7]. A small number of case reports have suggested a favorable response to cytarabine [4, 8], and in 1 patient to acyclovir [9]. However, it is unclear why some patients are able to mount a response to the JC virus causing PML [10] and thereby arrest further deterioration. This creates difficulty in evaluating the efficacy of potential treatments for this disease.

Once a rare disease, PML is becoming increasingly recognized in association with acquired immunodeficiency syndrome (AIDS) [7], with an estimated incidence of PML among AIDS patients as high as 4% [7]. The therapeutic management of these patients is empirical at the present time, although the successful use of cytarabine in AIDS patients has been reported [11].

Case History

A 37-year-old engineer was admitted with a 3-week history of progressive weakness of the left upper limb. His medical history was unremarkable. Although a smoker of 30 cigarettes a day he had no respiratory symptoms. On examination, he was fully alert and oriented. The significant findings were increased tone in the left arm and to a lesser extent in the leg, with mild proximal weakness in a pyramidal distribution, greater in the left upper than the lower limb. Reflexes were brisk on the left with an extensor plantar response in the left foot. Sensory examination revealed normal findings. Full blood cell count, erythrocyte sedimentation rate (ESR), electrolyte levels, and liver function were normal. A chest x-ray film showed bilateral hilar lymphadenopathy, with nodular shadowing in alveoli and band shadows in the lungs. Pulmonary function tests showed a mild restrictive pattern. Results of Mantoux tests of 1 in 10,000 and 1 in 1,000 were negative. A transbronchial biopsy confirmed the radiological suspicion of sarcoidosis in showing noncaseating granulomas. Bronchial alveolar lavage fluid contained 54% lymphocytes, with 45% macrophages, and a CD4/CD8 ratio of 2.5 : 1, consistent with active pulmonary sarcoidosis. A computed tomographic (CT) scan of the head showed patchy low-density lesions in the right posterior frontal and anterior parietal regions confined to white matter. A magnetic resonance image (MRI) (Fig 1) showed similarly sited lesions, without enhancement after gadolinium administration (Fig 2). A lumbar puncture was performed and the cerebrospinal fluid (CSF) pressure was 21 cm of CSF, with a protein level of 40 mg/

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