Modulation of Blood-Brain Barrier Dysfunction and Neurological Deficits during Acute Experimental Allergic Encephalomyelitis by the N-Methyl-D-aspartate Receptor Antagonist Memantine

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ABSTRACT
Previous studies by us have strongly indicated a role for the N-methyl-D-aspartate (NMDA) receptor in the pathogenesis of experimental allergic encephalomyelitis (EAE) and, moreover, the loss of blood-brain barrier (BBB) integrity implicit in the disease. The current investigation has used the NMDA receptor antagonist memantine to modify the neurological course of EAE and, in particular, prevent BBB breakdown. Memantine was administered orally either semiprophylactically, from day 7 postinoculation (PI), or therapeutically, 10 to 11 days PI. Semiprophylactic administration of drug at 60 mg/kg b.wt. significantly restored BBB integrity, reduced symptoms, and limited inflammatory lesions (p < 0.05), when assessed 12 days PI. Higher concentrations of memantine did not notably advance disease improvements observed at 60 mg/kg b.wt., and 40-mg/kg b.wt. doses only reduced histological scores (p < 0.05). Therapeutic application of memantine was found to be as effective as semiprophylactic dosing. Administration of drug at 60 mg/kg b.wt. was demonstrated as the optimum dose, significantly reducing disease, BBB permeability, and lesions (p < 0.01). Extended studies revealed that, after cessation of memantine treatment using either dosing regime, any subsequent appearance of disease was suppressed in severity and duration. We have provided further strong evidence in support of a role for the NMDA receptor in the development of EAE and, in particular, the loss of BBB function and recruitment of inflammatory cells. Moreover, memantine is therapeutically efficacious, suggesting the NMDA receptor as a viable pharmacological target for future treatment of human neurological conditions such as multiple sclerosis.

Dysfunction of the blood-brain barrier (BBB) is a pathological feature common to both multiple sclerosis (MS) and the animal counterpart, experimental allergic encephalomyelitis (EAE) (Moor et al., 1994). Under normal conditions the neurovasculature tightly regulates entry of solutes and cells of the immune system into the cerebral compartment (Bolton, 1997). However, with the onset of EAE and during the development of new MS lesions BBB integrity is functionally impaired (Hawkins et al., 1992; Moor et al., 1994). The resulting unregulated entry of cells and plasma constituents into central nervous system (CNS) tissues disturbs homeostasis within the neuronal environment and leads to the development of inflammatory lesions.

Although the precise mechanisms mediating the onset of BBB dysfunction are unknown, many contributory factors from inflammatory, endothelial, and neuronal cell sources have been identified (Bolton, 1997). Those factors proposed as causal mediators of neurovascular dysfunction during EAE include the cytokines tumor necrosis factor-α and interleukin-1 (Royall et al., 1989), histamine (Orr and Stanley, 1989), arachidonic acid metabolites (Ohnishi et al., 1992), free radicals (Shukla et al., 1996), and products of the ornithine decarboxylase cascade (Koenig et al., 1989). Whether loss of BBB function in MS and EAE is a primary insult in disease development or secondary to immune cell activation the abnormality is clearly an important pharmacological target, and measurement of neurovascular permeability remains a robust indicator of drug efficacy (Paul and Bolton, 1995; Bolton and Paul, 1997).

ABBREVIATIONS: BBB, blood-brain barrier; MS, multiple sclerosis; EAE, experimental allergic encephalomyelitis; CNS, central nervous system; NMDA, N-methyl-D-aspartate; PBS, phosphate-buffered saline; PI, postinoculation; EVBE, extravascular blood equivalent; MK-801, dizocilpine maleate.
Our studies have recently identified an overactivation of the N-methyl-D-aspartate (NMDA) receptor as a novel mechanism contributing to BBB dysfunction during EAE (Bolton and Paul, 1997; Paul and Bolton, 1998). In support of our findings, previous analysis of CNS tissues isolated from EAE-diseased animals has found elevated levels of the endogenous excitatory amino acids glycine and quinolate, which act as NMDA receptor agonists (Honegger et al., 1989; Flanagan et al., 1995). Interestingly, involvement of the NMDA subtype of glutamate receptor has been strongly implicated in a number of neurodegenerative conditions including Alzheimer’s disease, Huntington’s disease, Parkinson’s disease (Parsons et al., 1998), and more recently in MS (Bolton and Paul, 1997; Paul and Bolton, 1998). Many published articles link the NMDA receptor with BBB permeability in nondisease models. In particular, supporting evidence by Koenig and coworkers in an experimental cold injury model of BBB dysfunction also demonstrates involvement of the NMDA receptor in neurovascular breakdown via the downstream production of polyamines (Koenig et al., 1989, 1992). Furthermore, the group has reported the presence of NMDA receptors at the BBB after binding studies with the specific antagonist MK-801 (Koenig et al., 1992).

Pharmacological investigations by us in EAE strongly suggest subsequent involvement of the NMDA receptor in BBB dysfunction and subsequent disease expression. Administration of MK-801 significantly reduced neurovascular permeability after prophylactic and therapeutic treatments concomitant with improved neurological status (Bolton and Paul, 1997). Moreover, we have shown that NMDA receptor-dependent nitric oxide and polyamine production are elevated at the time of disease expression and can be modulated by administration of MK-801 (Paul et al., 1997). The location of the NMDA receptor population involved in neurovascular opening and hence disease progression in EAE is unknown but may be situated at neurovascular sites (Koenig et al., 1992; Stăsny et al., 2002), on the mast cell population activated during disease development (Bø et al., 1991; Purcell et al., 1996), or in association with neurons as in Huntington’s and Parkinson’s diseases (Parsons et al., 1998, 1999).

The aminoadamantane memantine is an NMDA receptor antagonist, structurally distinct from MK-801, which blocks receptor activation by binding to an ion channel site, presumed specific for phencyclidine (Parsons et al., 1999). Importantly, the aminoadamantanes seem well tolerated, in comparison with MK-801, a feature attributed to their kinetics at the NMDA receptor (Kornhuber et al., 1994). Indeed, memantine and parent compound amantadine have been used clinically with good tolerability for over 15 years, including use in dementia and as second-line drugs for the treatment of Parkinsonism (Young et al., 1997; Parsons et al., 1999). Furthermore, and consistent with our proposed role for the NMDA receptor in EAE-induced BBB dysfunction, memantine has been shown to modulate aspects of neurological disease development in acute EAE (Wallström et al., 1996). We now present the results of a comprehensive study analyzing the efficacy of memantine on the control of EAE disease expression and pathology in the Lewis rat. The work examines both semiprophylactic and therapeutic application of the compound by measuring changes in BBB permeability, histological profiles, and observing the appearance and severity of neurological deficits during and after cessation of drug administration.

Materials and Methods

Animals. Male Lewis rats, 7 to 9 weeks old, were used from stock bred on site, and housed five animals per cage, with food (Labsure CRM diet; Special Diet Services, Witham, Essex, UK) and water ad libitum. All procedures were carried out in accordance with the 1986 Animal Scientific Procedures Act and under Home Office approved project and personal licenses.

Induction of EAE. EAE was induced in animals as described previously (Paul and Bolton, 1998). Briefly, an emulsion comprising equal parts of guinea pig spinal cord, sterile phosphate-buffered saline (PBS), and incomplete Freund’s adjuvant (Difco, Detroit, MI) was prepared and supplemented with 10 mg ml⁻¹ Mycobacterium tuberculosis H₃⁷Rv (Difco). Rats were inoculated with 0.1 ml of inoculum into each hind footpad. A minimum of five animals was used per treatment.

Evaluation of Neurological Deficits. Animals were weighed daily and assessed for neurological disease beginning 10 days post-inoculation (PI). Disease symptoms were scored as follows: 1, flaccid tail; 2, hind limb hypotonia; 3, partial hind limb paralysis; and 4, complete hind limb paralysis.

Assessment of Histological EAE. The cervical spinal cords of animals from vehicle and drug treatments, sampled on day 12 PI, were examined by light microscopy for inflammatory lesions. Cervical tissue was selected for analysis because a heavy lesion load can be guaranteed during early EAE (Bolton et al., 1984), and therefore compound efficacy to restrict lesion development could be confidently evaluated.

A 1-cm length of cervical spinal cord was dissected and snap frozen. Tissue sections were cut at 5-μm thickness at one standard depth and stained with hematoxylin and eosin. Lesion number per section was quantitated “blind”. Each section was also assessed for increasing intensity of cellular infiltration on a scale from 0 to 4.

Preparation and Administration of Memantine. Memantine (Sigma-Aldrich, Poole, Dorset, UK) was dissolved in sterile PBS at a concentration of 20 mg/ml and administered orally either semiprophylactically or therapeutically. EAE-sensitized animals receiving memantine semiprophylactically were dosed once daily with 40, 60, or 80 mg/kg b.wt. for 6 days beginning day 7 PI, which corresponds with mid-effector phase of disease development. Animals treated therapeutically received the drug once daily at 20, 40, 60, or 80 mg/kg b.wt. on days 10 and 11 PI. Control EAE-inoculated rats were administered an equivalent volume of sterile PBS vehicle alone.

Quantitation of BBB Integrity. BBB permeability in selected areas of the CNS was determined according to our previous methods (Paul and Bolton, 1995), which are detailed briefly below.

Labeling of Red Blood Cells with 111In-tropolonate. Cell-free plasma was prepared by repeated centrifugation of pooled Wistar rat blood. Blood cells from the primary centrifugation stage were resuspended in HEPES saline buffer (20 mM HEPES (Invitrogen, Carlsbad, CA) and 0.8% NaCl), repeatedly washed to remove leukocytes, and reconstituted to provide an erythrocyte concentration of 5 × 10⁸ cells ml⁻¹. The cell preparation was incubated at 37°C for 20 min with 2 μCi of 111In-tropolonate/5 × 10⁸ red blood cells, followed by washing and resuspension at 5 × 10⁶ cells/0.5 ml of cell-free plasma.

Determination of BBB Permeability. Rats received 10 μCi of 125I-labeled serum albumin intravenously under halothane/oxygen anesthetic and, 24 h later, 5 × 10⁹ 111In-red blood cells were injected as a blood volume marker. Cardiac blood was collected, after a 4.5-min circulation time, into heparin-coated tubes followed by a lethal injection of Euthatal (RMB Animal Health Ltd., Dagenham, UK) at 5 min. Cerebella, medulla pons, and cervical spinal tissues were dissected and the 111In levels in tissue samples, and 100-μl blood aliquots from each animal were recorded using a minigamma counter (LKB, Uppsala, Sweden). The 125I content of samples was...
assessed after $^{111}\text{In}$ decay after storage for 3 weeks at $-20^\circ\text{C}$. BBB permeability, expressed as extravascular blood equivalents (EVBEs), was calculated from isotope levels in tissue and blood (eq. 1) and is a measure of radiolabeled albumin crossing the neurovasculature with accumulation in CNS tissues.

$$\frac{125\text{I Tissue cpm/g}}{111\text{In Tissue cpm/g}} \times \frac{111\text{In Blood cpm/ml}}{125\text{I Blood cpm/ml}} \times 100 = \text{EVBE}$$

Corticosterone Radioimmunoassay. Circulating corticosterone levels in rats were determined to exclude the possibility that the treatment regimes used enhanced endogenous steroid levels, which are known to influence the course of EAE (MacPhee et al., 1989) and could therefore contribute to drug efficacy. Blood was collected into heparin-coated tubes, at a standard time, by cardiac puncture and plasma separated by centrifugation at 300g with subsequent storage at $-20^\circ\text{C}$ before assay. Plasma corticosterone levels were measured in samples from all efficacious drug treatments using a $\gamma$-B $^{125}\text{I}$ corticosterone radioimmunoassay kit (IDS, Tyne and Wear, UK) and according to the manufacturer’s instructions.

Statistical Analysis. Differences in results from drug and vehicle treatments were determined using the Mann-Whitney $U$ test for nonparametric data with Bonferroni’s correction for multiple comparisons where required.

Results

Quantitation of BBB Dysfunction on Day 12 PI

BBB permeability was assessed in EAE-inoculated rats on day 12 PI and corresponding with the end of memantine treatment. Our previous studies have conclusively shown that the time point coincides with the development of neurological deficits and neurovasculature dysfunction in the pre-selected CNS regions analyzed (Paul and Bolton, 1995). Typical EVBE values were obtained from normal rats and control EAE-sensitized animals receiving vehicle alone (Fig. 1A). Characteristically, spinal and medulla pons tissues exhibited greater permeability compared with the cerebella on day 12 PI as reported previously by us (Paul and Bolton, 1995; Bolton and Paul, 1997).

Effect of Semiprophylactic Dosing with Memantine on BBB Permeability and Neurological EAE. Treatment with memantine, at 40 mg/kg b.wt., failed to improve either neurovascular permeability (Fig. 1A) or neurological deficits (Fig. 1B). In contrast, administration of memantine at 60 mg/kg b.wt. caused a significant suppression of BBB opening in all tissues (Fig. 1A; $p < 0.05$), and a concomitant inhibition of neurological symptoms (Fig. 1B; $p < 0.05$). However, a further improvement in BBB function and neurological status was not achieved by increasing the drug dose to 80 mg/kg b.wt.

Effect of Therapeutic Treatment with Memantine on BBB Permeability and Neurological EAE. The marked differences between normal levels of BBB permeability and values for vehicle-treated EAE-sensitized controls were reconfirmed (Fig. 2A). Memantine, when administered therapeutically at 20 mg/kg b.wt., was ineffective at preventing abnormal BBB opening and inhibiting neurological signs (Fig. 2, A and B). However, and in contrast to results obtained using a semiprophylactic regime, administration of drug at 40 mg/kg b.wt. significantly improved neurovascular function in the cerebellum (Fig. 2A; $p < 0.05$) and markedly curtailed the development of neurological deficits (Fig. 2B; $p < 0.01$). Memantine was most effective when given at 60 mg/kg b.wt., as evidenced by a dramatic improvement in BBB integrity in all sampled CNS regions (Fig. 2A; $p < 0.01$) and complete suppression of disease symptoms (Fig. 2B; $p < 0.001$). An increase in drug concentration to 80 mg/kg b.wt. did not further improve BBB function or neurological status.

Histological Assessment of CNS Lesions on Day 12 PI

Presence of CNS Lesions after Semiprophylactic Administration of Memantine. Lesion intensity in the CNS of vehicle-treated EAE-sensitized rats was not significantly different from values recorded in spinal tissue from undosed inoculated control animals (Table 1). Spinal tissues from EAE-sensitized animals receiving memantine for 6 days before sampling showed significant reductions in the intensity and frequency of lesions at all doses of drug ($p < 0.05$). Interestingly, the reduced lesion load in spinal tissues from animals receiving memantine at 40 mg/kg b.wt. was not reflected in an improvement of either BBB permeability or neurological status.

Presence of CNS Lesions after Therapeutic Memantine. Vehicle-treated EAE-sensitized rats had a similar mean lesion number to that recorded for undosed inoculated animals (Table 1). Therapeutic administration of memantine at 60 mg/kg b.wt. significantly suppressed the intensity ($p <
and remained suppressed for the duration of the experiment (Table 2).

Extended Efficacy of Therapeutic Memantine. Memantine was evaluated at concentrations of 40 and 60 mg/kg b.wt., which had demonstrated submaximum and maximum efficacy, respectively, in the ability to suppress disease 12 days PI (Figs. 1 and 2; Table 1). Low-dose memantine completely inhibited symptoms in six of seven animals between 10 and 13 days PI (Fig. 4A) with a transient, mild episode of disease emerging in six rats, 14 to 17 days PI (Table 2). Treatment with memantine at 60 mg/kg b.wt. significantly reduced symptoms 12 to 14 days PI \( (p < 0.05) \), followed by an emergence of low-grade disease in four of seven animals (Fig. 4B; Table 2).

Body Weight Profiles

Figure 5, A and B, illustrates the body weight profiles of animals treated semiprophylactically and therapeutically with memantine. The data from control groups show the typical weight loss associated with the development of neurological EAE, starting approximately 10 days PI and continuing until the complete regression of symptoms. The results also show an initial drug-associated weight loss 1 day after commencement of semiprophylactic (Fig. 5A) and therapeutic (Fig. 5B) dosing, which is not repeated on succeeding days, suggesting tolerance of the treatment.

Endogenous Corticosterone Levels

Plasma samples collected from undosed and vehicle-treated rats contained characteristically elevated levels of endogenous corticosterone associated with EAE development (Table 3; MacPhee et al., 1989). Semiprophylactic and therapeutic treatment of EAE-inoculated rats with memantine at 40 and 60 mg/kg b.wt. did not induce an elevation in circulating corticosteroids above control levels. Indeed, the dosing regimes corresponded with reduced corticosterone levels, compared with vehicle controls, and an overall improvement in the neuropathological status of the animals.

Corticosteroid levels in rats treated with memantine at 80 mg/kg b.wt. remained similar to vehicle controls despite a significant reduction in the parameters of EAE analyzed. Therefore, the use of memantine at 80 mg/kg b.wt. seems associated with a rise in endogenous steroid production and, together with body weight loss in excess of control animals (data not shown), suggests dose-dependent, detrimental drug-induced effects.

The results confirm semiprophylactic and therapeutic administration of memantine at 40- and 60-mg/kg b.wt. does not increase endogenous corticosteroid levels above vehicle control values. Therefore, the modification of BBB permeability, CNS inflammatory cell influx, and neurological deficits after memantine administration does not result from a drug-induced elevation in circulating endogenous immunomodulatory glucocorticoids.

### Discussion

The noncompetitive NMDA receptor antagonist memantine effectively reduced BBB permeability, the development and duration of neurological deficits, and the accumulation of CNS inflammatory infiltrates when used in either semipro-
dosing period, associated with a semiprophylactic regime, active amounts of memantine accumulate over the prolonged nation for the disparity of effects is that pharmacologically infiltration when given therapeutically. One possible expla-
lar dysfunction, attenuated lesion development. Conversely,
wise ineffective in reducing signs of disease and neurovascu-
permeability. Semiprophylactic low-dose memantine, other-

variable results between the two dosing regimes, which may
The lower dose of 40 mg/kg b.wt. memantine demonstrated
in cervical spinal tissue was dose-related after therapeutic
regimes. Similarly, the inhibition of neurovascular damage
reduction in neurological scores was recorded using both
therapies. However, memantine at a dose of 60 mg/kg b.wt.,
inflammatory infiltrates, which is in contrast to the studies
of Wallström et al. (1996) who found i.p. memantine to be ineffective at suppressing lesion development. Our current
findings and the previous observations (Wallström et al.,
1996) used different routes of drug administration, which
may account for the variability in efficacy. Interestingly,
preliminary studies by us using an i.p. dosing regime compa-
reducible drug efficacy.

Studies by Wesemann et al. (1982) indicate the oral route
for memantine administration is superior to other routes for
maintaining drug levels in CNS tissues. Oral memantine
achieves maximal brain concentration 1 h after dosing and
plateaus for a minimum of 4 h. In contrast, i.p. administra-
tion of memantine generates a peak drug concentration at
1 h, but rapidly decreases without establishing the steady
state achieved by oral dosing (Wesemann et al., 1982). Pre-
vious investigations by us have indicated that oral dosing of
compounds is less stressful than the i.p. route, which can
exert a suppressive influence on the course of EAE through
elevation of endogenous steroid levels (Scott et al., 1996).
Furthermore, demonstration of oral activity offers greater
clinical potential due to ease of administration and reflects
currently employed formulations of the drug as used for
therapy in Parkinson’s disease (Wesemann et al., 1982; Young et al., 1997).

The current study provides further compelling evidence for
an involvement of the NMDA subtype of glutamate receptor
in the progression and development of acute EAE in the
Lewis rat. Importantly, the equipotent efficacy of semipro-

Fig. 3. Mean neurological scores (± S.E.M.) during acute EAE and after semiprophylactic treatment with memantine. Memantine was admin-
tered at 60 mg/kg from day 7 to 12 PI. Neurological signs were recorded
until the recovery of control EAE groups at day 17 PI. *, p < 0.05, significantly different compared with vehicle treatment.

Table 1: Histopathology of the cervical spinal cord 12 days PI and after treatment with memantine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Lesion Intensity</th>
<th>Mean Lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Undosed EAE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>80 mg/kg b.wt. memantine</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>60 mg/kg b.wt. memantine</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>40 mg/kg b.wt. memantine</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Therapeutic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7</td>
<td>0</td>
</tr>
<tr>
<td>60 mg/kg b.wt. memantine</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>40 mg/kg b.wt. memantine</td>
<td>7</td>
<td>3</td>
</tr>
</tbody>
</table>

a,b Combined results from two studies.

c Lesion intensity, p < 0.05, significantly different from vehicle control.
d Lesion number, p < 0.05, significantly different from vehicle control.
phylactic and therapeutic dosing at the optimum dose of 60 mg/kg b.wt. suggests NMDA receptor involvement is not at the induction phase of EAE, but rather coincident with or slightly before neurological symptoms and BBB breakdown (Bolton and Paul, 1997). Clearly, there is no enhanced suppression of symptoms using a prophylactic dosing regime, which indicates drug action conterminous to disease onset.

Importantly, the therapeutic efficacy of memantine supports use of the drug during the onset of clinical episodes in human CNS conditions, including the demyelinating diseases.

Location of the NMDA receptor at neuronal sites is well documented (Parsons et al., 1998), and the receptor has now been implicated on brain- and peritoneal-derived mast cells (Purcell et al., 1996). Activation of the mast cell NMDA receptor causes degranulation and the release of histamine, a recognized mediator of neurovascular permeability (Dux and Joë, 1982; Purcell et al., 1996). Elevated CNS histamine levels and degranulated mast cells have been detected at the onset of EAE and BBB breakdown and are therefore coinci-

**TABLE 2**

Effect of memantine on the progression of neurological disease in EAE after cessation of treatment

<table>
<thead>
<tr>
<th></th>
<th>No. of Diseased Animals/Total</th>
<th>Disease Onset(^a,b)</th>
<th>Duration(^a,b)</th>
<th>Peak Neurological Score(^a,b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Semiprophylactic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>6/8</td>
<td>11 ± 0.2</td>
<td>5.2 ± 0.6</td>
<td>2.8 ± 0.5</td>
</tr>
<tr>
<td>60 mg/kg b.wt. memantine</td>
<td>5/8</td>
<td>12 ± 0.7</td>
<td>2.8 ± 0.5(^c)</td>
<td>1.4 ± 0.4</td>
</tr>
<tr>
<td>40 mg/kg b.wt. memantine</td>
<td>7/9</td>
<td>13 ± 0.6(^c)</td>
<td>3.1 ± 0.7</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Therapeutic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>7/8</td>
<td>12 ± 0.6</td>
<td>4.9 ± 0.7</td>
<td>2.9 ± 0.4</td>
</tr>
<tr>
<td>60 mg/kg b.wt. memantine</td>
<td>5/7</td>
<td>15 ± 0.5(^c)</td>
<td>2.0 ± 0.5(^c)</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>40 mg/kg b.wt. memantine</td>
<td>6/7</td>
<td>14 ± 0.7</td>
<td></td>
<td>1.8 ± 0.3</td>
</tr>
</tbody>
</table>

\(^{a}\) Mean values ± S.E.M. recorded for treatment groups assessed between days 10 and 18 PI.

\(^{b}\) Mean values recorded for animals exhibiting neurological signs of disease.

\(^{c}\) \(p < 0.05\) significantly different from vehicle control.
dent with our proposed actions of memantine in the disease (Orr and Stanley, 1989; Stanley et al., 1990; Bø et al., 1991; Rouleau et al., 1997). Accordingly, the action of memantine during EAE may include the prevention of mast cell degranulation as reported previously for isolated cell studies with NMDA receptor antagonists (Purcell et al., 1996).

Previous studies indicate that NMDA receptors involved in the development of BBB dysfunction, and by implication in early neurological EAE, are located at neurovascular sites. Koenig et al. (1992) presented indirect evidence of NMDA receptor location at neuroendothelial sites through binding studies with CNS microvascular preparations and the channel blocker MK-801, which has recently been supported by studies with CNS microvascular preparations and the channel blocker MK-801 (Staastyn et al., 2002) using both binding studies and analysis of mRNA expression. The study by Koenig et al. (1992) attributed NMDA receptor modulation of neurovascular function to the direct and subsequent activation of ornithine decarboxylase, the precursor enzyme responsible for the production of the polyamines putrescine, spermidine, and spermine (Koenig et al., 1992). Interestingly, our investigations in acute EAE have shown profound alterations in the levels of the polyamines at disease onset and BBB breakdown that are sensitive to MK-801 administration (Bolton et al., 1994; Paul et al., 1997), again implicating NMDA receptor-mediated mechanisms in the pathogenesis of EAE.

The importance of the well characterized neuronal NMDA receptors in the pathology of acute EAE and associated BBB dysfunction has, to our knowledge, not been considered. However, there is clear evidence for the role of NMDA receptor-stimulated neurons in other neurodegenerative diseases such as Alzheimer’s and Parkinsonism (Parsons et al., 1998). Therefore, it is reasonable to suggest that the NMDA receptor population of neurons may also have a role in MS and EAE. In support of our suggestions, intraventricular, intrathecal, and intrastriatal administration of NMDA to rats has led to the induction of BBB permeability, which is intrinsic to the pathology of MS and EAE (Dietrich et al., 1992; Nag, 1992; Miller et al., 1996). Importantly, the neuronal α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid glutamate receptor has recently been closely linked with neuronal loss and oligodendrocyte depletion during EAE (Pitt et al., 2000; Smith et al., 2000).

Potent antagonists of the NMDA receptor, such as MK-801, are invariably associated with the induction of psychotomimetic effects (Tricklebank et al., 1989). In contrast, the aminodamantanes are low-affinity antagonists of the NMDA receptor and are reportedly better tolerated as a result of fast voltage-dependent channel unblocking kinetics (Parsons et al., 1999). Crucially, memantine seems to differentiate between transient physiological activation and sustained pathological activation of the NMDA receptor, acting primarily under the latter conditions (Parsons et al., 1999). Current theory suggests that memantine, like Mg²⁺, will vacate the receptor channel rapidly under strong synaptic depolarization as encountered during physiological activation by millimolar concentrations of glutamate. However, in contrast to Mg²⁺, memantine will maintain channel block during prolonged depolarization triggered by sustained micromolar concentrations of glutamate under pathological conditions (Parsons et al., 1999). The success of memantine in the current study, after an oral dosing regime, further supports the potential for the wider use of the group of compounds in the clinical setting.

In summary, the study has shown memantine to be an effective agent for the control of neurological disease in EAE. The drug significantly restricted BBB permeability, reduced inflammatory infiltrates into the CNS, and eliminated neurological symptoms. Furthermore, on discontinuation of treatment the severity and duration of the subsequent disease episode were markedly limited. Our findings strongly reinforce a role for the NMDA receptor in acute EAE and reemphasize the close association of the glutamate subtype receptor with the loss of neurovascular integrity. Further study of the mechanism of action of NMDA receptor involvement in BBB dysfunction and neuroinflammation may elucidate new targets for therapy. Moreover, identification of further well tolerated NMDA receptor antagonists, acting either at specific modulatory sites or to block channel function, could reveal novel neuroprotective treatments for human degenerative diseases such as MS.

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References


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