

## Riluzole, a Novel Neuroprotective Agent, Attenuates Both Neurologic Motor and Cognitive Dysfunction Following Experimental Brain Injury in the Rat

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### ABSTRACT

Several potential mechanisms are involved in mediating the pathophysiology of traumatic brain injury (TBI), including inflammatory processes and excitotoxicity. In the present study, we evaluated the ability of the use-dependent sodium channel inhibitor Riluzole to attenuate cognitive and neurologic motor deficits and reduce regional cerebral edema and histologic cell damage following lateral fluid-percussion (FP) brain injury in rats ( $n = 109$ ). In study 1, 58 anesthetized male Sprague-Dawley rats (350–400 g) were subjected to FP brain injury of moderate severity (2.3–2.5 atm). Fifteen minutes following brain injury, animals randomly received an i.v. bolus of either Riluzole (4 mg/kg,  $n = 11$ ), Riluzole (8 mg/kg,  $n = 11$ ), or glycol vehicle ( $n = 20$ ), followed by 6 h and 24 h s.c. injections (identical dose). Surgically prepared but uninjured animals received vehicle ( $n = 16$ ) and served as controls. Animals were evaluated for cognitive deficits at 48 h postinjury and killed for assessment of regional brain edema. Administration of vehicle or Riluzole (4 mg/kg  $\times$  3) had no significant effect on memory or edema, whereas Riluzole (8 mg/kg  $\times$  3) significantly attenuated post-traumatic cognitive dysfunction ( $p < 0.05$ ). In study 2, a second group of animals ( $n = 25$ ) was injured, treated with Riluzole (8 mg/kg  $\times$  3 doses,  $n = 13$ ) or vehicle ( $n = 12$ ), and evaluated for neurologic motor function over 2 weeks. Animals treated with Riluzole demonstrated significantly improved motor scores beginning 1 week postinjury ( $p < 0.05$ ). In study 3, brain-injured animals were treated with Riluzole (8 mg/kg  $\times$  3 doses,  $n = 10$ ) or vehicle ( $n = 10$ ), and posttraumatic lesion volume was assessed at 48 h postinjury using 2,3,5-triphenyltetrazolium chloride (TTC) staining. Treatment with Riluzole had no significant effect on posttraumatic lesion volume. The present study demonstrates that use-dependent sodium channel inhibitors, such as Riluzole, can attenuate both cognitive and neuromotor dysfunction associated with brain trauma.

**Key words:** brain injury; cognition; edema; excitotoxicity; glutamate; neuroprotection; rat

### INTRODUCTION

**T**RAUMATIC BRAIN INJURY (TBI) is the major cause of mortality and morbidity in young people between the

ages of 15 and 24 years worldwide, with incidence rates ranging from 150 to 400 per 100,000 per year requiring hospitalization (Kraus, 1995). The cascade of delayed or secondary pathologic events that follows an injury is ex-

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tremely complex, and the relative importance of each event appears to differ in each individual case. Therefore, a great need exists for a broad-spectrum neuroprotective agent that may target several pathophysiological processes and thus confer widespread protection to the damaged brain. Riluzole, a novel compound whose properties have been described in detail previously (Stutzmann and Doble, 1995), has been shown to be neuroprotective in a number of experimental models of cerebral ischemia and neurodegenerative diseases (Malgouris et al., 1989; Pratt et al., 1992; Boireau et al., 1994; Benazzouz et al., 1995). Riluzole can suppress *in vitro* hyperexcitability of both cultured cerebellar granule cells and hippocampal slices (Mizoule et al., 1985; Stutzmann et al., 1991; Doble et al., 1992) and also appears to stimulate repair mechanisms of cells that are injured *in vitro* by irradiation (Alaoui et al., 1995).

*In vivo*, Riluzole is a potent anticonvulsant, with several studies demonstrating protective effects in models of epileptic seizures (Mizoule et al., 1985; Stutzmann et al., 1991). This compound has been shown to improve EEG activity, reduce infarct volume, and prevent memory loss and hippocampal CA1 pyramidal cell loss after ischemic brain injury in rat and gerbil (Malgouris et al., 1989; Pratt et al., 1992). Riluzole treatment has been shown recently to stimulate the return of somatosensory evoked potentials and reduce the extent of spinal cord infarct in a model of spinal cord injury in rats (Stutzmann et al., 1996). Treatment with Riluzole has also been shown to partially antagonize the MPP(+)-induced increase in striatal extracellular dopamine in rats (Boireau et al., 1994) and significantly reduce parkinsonian motor symptoms (particularly bradykinesia and rigidity) in the nonhuman primate (Benazzouz et al., 1995). These encouraging experimental data led to the clinical examination of Riluzole in amyotrophic lateral sclerosis (ALS), a motoneuron disease causing chronic and progressive degeneration followed by paralysis and death. Riluzole treatment produced a statistically significant displacement of the survival curve (Bensimon et al., 1994).

One component of the secondary neurochemical response to traumatic CNS injury appears to include an acute and prolonged release of excitatory amino acid neurotransmitters (EAA), with concomitant pathologic overexcitation of EAA subtype receptors. Using intracerebral microdialysis techniques, a marked and acute increase in extracellular EAA concentrations has been reported to occur within minutes following experimentally induced TBI in rodents (Faden et al., 1989; Katayama et al., 1990; Nilsson et al., 1990; Panter and Faden, 1992; Palmer et al., 1993). This pathologic increase in glutamate release following brain trauma is accompanied by a widespread depolarization and concomitant increase in

extracellular potassium concentrations, which can further contribute to the pathologic release of EAAs (Katayama et al., 1990; Faden, 1993; McIntosh, 1994).

Recently, compounds that can attenuate sodium-induced glutamate release have been shown to improve neurobehavioral outcome in experimental models of brain trauma (Okiyama et al., 1995; Sun and Faden, 1995). In the present study, we comprehensively evaluated the therapeutic efficacy of Riluzole with respect to posttraumatic regional cerebral edema, neurologic motor deficits, cognitive function, and posttraumatic lesion volume following experimental lateral fluid-percussion (FP) brain injury in the rat.

## MATERIALS AND METHODS

### Experimental Brain Injury

Male Sprague-Dawley rats (350–400 g,  $n = 109$ ) were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and placed in a stereotaxic frame. The scalp and temporal muscle were reflected, and a 3.5-mm hollow female Luer-Lok fitting was rigidly fixed with dental cement in to a craniotomy centered above the left parietal cortex, 5 mm anterior to lambda, 5 mm posterior to bregma, and 4 mm lateral to the sagittal suture. The dura was left intact at this opening. Ninety minutes after pentobarbital administration, lateral (parasagittal) experimental brain injury was induced using an FP device as previously described (McIntosh et al., 1989a). Briefly, the animal is connected to the FP injury device via the female Luer-Lok fitting. The device produces a pulse of increased intracranial pressure of 21–23 msec duration through the rapid injection of saline into the closed cranial cavity, resulting in brief but profound displacement and deformation of the brain. This pressure pulse is measured extracranially by a transducer (Gould, Inc.) housed in the injury device and displayed on a computer oscilloscope. The pressure pulse is recorded in atmospheres (atm), with animals receiving brain injury of moderate severity (2.3–2.5 atm). Control sham animals ( $n = 16$ ) received identical anesthesia and surgical preparation without FP brain injury.

### Study 1: Evaluation of Cognitive Function

Evaluation of posttraumatic memory function was performed using an adaptation of the Morris water maze (MWM) paradigm (Morris, 1984). The MWM used is a circular tank (1 m in diameter and 50 cm deep) filled to 25 cm with 18°C water, which is covered with styrofoam pieces to render the surface opaque. A stationary, submerged, and nonvisible platform 11.5 cm  $\times$  11.5 cm and 24 cm tall (1 cm below the surface) is placed in a site de-

## RILUZOLE AND BRAIN INJURY

terminated from a grid design of various zones. This grid design, constructed with a computerized video system (video-scan lab animal monitoring system, Omnitech Electronics, Inc., Columbus OH) is superimposed over the maze and viewed on a monitor. Two days before injury, animals ( $n = 48$ ) were trained in the MWM to locate the platform using external visual cues. Each animal was given 20 training trials over the 2-day training period, and 2.5 h after the final trial, all animals were anesthetized and subjected to FP brain injury or sham injury as described. Fifteen minutes after FP brain injury, animals randomly received either (1) a 1-ml i.v. (into the femoral vein) infusion of Riluzole (4 mg/kg), followed by s.c. injections of Riluzole (4 mg/kg) at 6 h and 24 h postinjury ( $n = 11$ ) or vehicle (identical dosing schedule,  $n = 11$ ; vehicle is a glycol preparation), or (2) Riluzole (8 mg/kg) followed by s.c. injections of Riluzole (8 mg/kg) at 6 h and 24 h postinjury ( $n = 11$ ) or vehicle (identical dosing schedule,  $n = 15$ ). Doses and treatment paradigms were based on consultation with Rhône-Poulenc Rorer scientists and on both preliminary and previously published studies. Sham (uninjured) animals that were anesthetized and surgically prepared without injury received Riluzole (8 mg/kg,  $n = 4$ ) or vehicle ( $n = 12$ ) (at times identical to those of the experimental groups) to evaluate the effect of these compounds on uninjured animals. Femoral dissection for drug injection produced no observed untoward effects on motor function in any experimental group.

Forty-two hours postinjury, animals were tested for their ability to remember the preinjury learned task in the MWM. The platform was removed, and the animals were given a 1-min test period in the MWM while the computerized-video unit recorded their swimming patterns. Each zone of the computerized grid design is ranked in a weighted fashion according to its proximity to the platform (Smith et al., 1991, 1994, 1995). This paradigm has been used with great success by us and others to evaluate cognitive deficits in various models of brain trauma (Okuyama et al., 1992; Smith et al., 1991, 1994; Smith et al., 1994). The assigned numbers are multiplied by the number of seconds spent in the corresponding zone and totaled. The ranked numbers are derived from a series of previously published studies designed to assess the behavioral characteristics of trained injured and noninjured animals searching for the platform (Smith et al., 1991). Escape latencies (time used initially to cross through the platform site) were also recorded.

### *Evaluation of Cerebral Edema*

For cerebral edema determination, animals from the cognition study receiving either Riluzole (4 mg/kg i.v. at 15 min followed by 4 mg/kg s.c. at 6 h and 24 h,  $n = 11$ ) or Riluzole (8 mg/kg i.v. at 15 min followed by 8

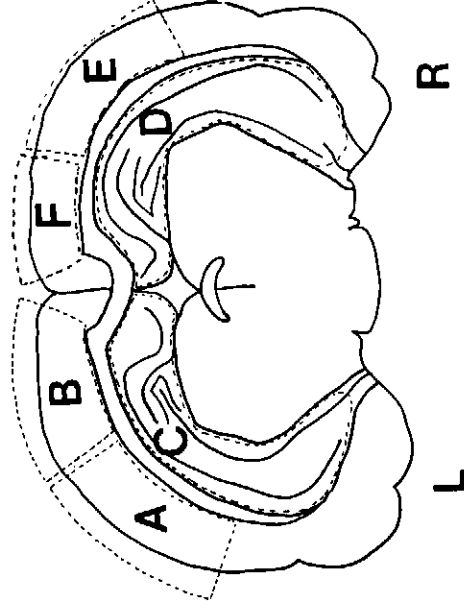
mg/kg s.c. at 6 h and 24 h,  $n = 11$ ) or vehicle (equal volume at 15 min, 6 h, and 24 h,  $n = 11$ ) were reanesthetized with sodium pentobarbital (200 mg/kg i.p.) at 48 h following FP brain injury and killed by decapitation. This time point has been previously shown to be the time of maximal edema formation in this model (Soares et al., 1992). Eight sham (uninjured) animals served as controls for brain water determinations. The brains were rapidly removed and dissected on a chilled marble plate into the following regions (Fig. 1): injured left parietal cortex (maximal injury site), cortex adjacent to maximal injury (adjacent site), contralateral right parietal cortex (contralateral cortex), bilateral hippocampus, and thalamus. Tissue samples were transferred to a cooled humid box. Brain water content was evaluated by the wet weight/dry weight technique: fresh tissue samples were weighed on aluminum foil, dried for 24 h at 100°C, and then reweighed. The percentage of water was calculated as

$$\left[ \frac{\text{Wet weight} - \text{dry weight}}{\text{wet weight}} \right] \times 100$$

In our laboratory, this technique has proven to be an extremely sensitive and reproducible method for the evaluation of regional cerebral edema formation after brain injury (Okuyama et al., 1992; Soares et al., 1992).

### *Study 2: Evaluation of Neurologic Motor Function*

Based on the initial positive results for cognitive function with the higher (8 mg/kg) dose of Riluzole, a second study was performed to evaluate the efficacy of this



**FIG. 1.** Schematic representation of brain regions dissected after experimental lateral fluid-percussion brain injury in the rat for analysis of regional cerebral edema. Region A is the site of maximal injury that occurs in the parietal/temporal cortex; region B is the cortex adjacent to the site of maximal injury; regions C and D encompass the ipsilateral (to the injury) and contralateral hippocampus, respectively; regions E and F are corresponding areas of the contralateral cortex. L, left; R, right.

dose on neurologic motor function. Animals were subjected to FP brain injury of moderate severity, as described. At 15 min postinjury, injured animals received an i.v. infusion of Riluzole (8 mg/kg) followed by s.c. injections of Riluzole (8 mg/kg) at 6 h and 24 h ( $n = 13$ ) or vehicle (equal volume,  $n = 12$ ). Evaluation of chronic posttraumatic motor dysfunction was assessed at 24 h, 48 h, 1 week, and 2 weeks postinjury by a trained observer who was unaware of each animal's treatment, using a battery of four composite neurologic motor function tests as previously described (Dixon et al., 1987; McIntosh et al., 1989a; Okiyama et al., 1992; Smith et al., 1993b). Animals are scored from 4 (normal) to 0 (severely impaired) for each of the following indices: (1) right (0-4) and left (0-4) forelimb flexion response during suspension by the tail (highest possible score for each side = 4), (2) decreased resistance to left (0-4) and right (0-4) lateral pulsion (highest possible score for each side = 4), (3) ability to traverse a narrow 1.5-m wooden beam to enter a darkened goal box (beam-walk task), where each animal was scored (0-4) by evaluating its ability to balance and remain on the beam, limb placement during movement, incidence of foot slippage, and body elevation relative to the beam, and (4) movement across a grid field both vertically and horizontally, as measured by a computerized activity monitor (Opto-Varimax, Columbus Instruments). Spontaneous locomotor activity, both stereotypic and ambulatory, is recorded, and activity scores are calculated: 89%-100% of baseline activity = 4, 78%-88% of baseline = 3.5, 67%-77% of baseline = 3, 56%-66% of baseline = 2.5, 45%-55% of baseline = 2, 34%-44% of baseline = 1.5, 23%-33% of baseline = 1, 12%-22% of baseline = 0.5, and 0-11% of baseline = 0. A total composite functional neurologic score (0-36) is obtained by combining the scores for each of the four neurobehavioral tests.

### Study 3: Characterization of Posttraumatic Lesion Volume

A third group of animals was subjected to FP brain injury of moderate severity as described. At 15 min postinjury, injured animals received an i.v. infusion of Riluzole (8 mg/kg), followed by s.c. injections at 6 h ( $n = 10$ ) or glycol vehicle (equal volume,  $n = 10$ ). At 48 h following FP brain injury, rats were reanesthetized with sodium pentobarbital (200 mg/kg, i.p.) and decapitated, and their brains were rapidly removed. Brains were immediately placed on a chilled stage, secured with agarose, and then refrigerated for 2 min. Each brain was then cut into 1-mm coronal sections using a McIlwain Tissue Chopper (Mickle Laboratory Engineering Co. LTD., Gornshell, Surrey, England). The brain sections were separated in a bath of 0.2 M phosphate buffer at 37°C. All agarose was

removed, and phosphate buffer was drained from the dish. The tissue sections were aligned in the Petri dish, immersed in 10 ml of a 37°C solution of 2% TTC (Sigma Chemical Co., St. Louis MO) prepared in 0.2 M phosphate buffer. Sections were immediately covered by a glass slide, light-protected by covering with foil, and stained for 7 min. (A series of preliminary studies in our laboratory demonstrated that a period of 7 min is necessary to achieve staining of viable tissue.) The glass slide (not a coverslip) was used because it was effective in ensuring consistent and reproducible staining in a short time. After staining, tissue sections were washed twice with 0.2 M phosphate buffer and stored in 10% neutral buffered formalin.

### Tissue Section Analysis

All imaging and lesion volume analysis were performed by an independent investigator blinded to whether the animal was injured (experimental) or sham (uninjured control), as well as to postinjury treatment status. Digitized monochrome images of the rostral side of each section were captured and stored (total of 4-10 sections per animal, corresponding to injury severity) using a Dage-MTI CCD72 video camera and M1-MCID imaging system software (Imaging Research, Brock University, St. Catharines, Ontario, Canada). Lesion area of each image was determined by enlarging the sections and using a standardized imaging scheme, which allowed for easier demarcation between red tissue (normal—suggesting high mitochondrial activity), pink tissue (damaged—suggesting reduced mitochondrial respiration), and white tissue (minimal or no mitochondrial activity). The white and pink tissue taken together was determined to be the area of lesion. In each of the 1-mm coronal brain sections with white and/or pink tissue, the lesion area was calculated. This lesion area was added to other serial sections with a lesion to give a sum (rostral-caudal) total lesion volume, expressed in cubic millimeters. Previous studies in our laboratory have demonstrated a correlation between posttraumatic TTC lesion volume and injury severity (Perré et al., 1995).

### Evaluation of Brain and Core Body Temperature

Because core brain temperature has been shown to correlate directly with temporalis muscle temperature (Corbett et al., 1990), a needle temperature probe (YSI Inc., Boston MA) was inserted into the right temporalis muscle of animals 1 h before brain injury or sham treatment. In addition, a rectal temperature probe was inserted 1 h before brain injury or sham treatment for measurement of core body temperature. Animals received a 1-ml i.v. injection of either Riluzole (8 mg/kg) at 15 min ( $n =$

4), or vehicle ( $n = 4$ ). Temperature readings from both probes were recorded every 15 min for 1 h following brain or sham injury and drug administration.

#### Data Analysis

All data are expressed as means  $\pm$  SEM, except for behavioral assessment scores (ordinal data), which are expressed as median values. Continuous variables are compared across groups by analysis of variance (ANOVA) followed by Student-Newman-Keuls test. Ordinal measurements, such as neurologic motor scores and memory scores, were evaluated using nonparametric Kruskal-Wallis ANOVA followed by individual Mann-Whitney U-tests. Statistical analysis to evaluate lesion volume was performed on the Riluzole-treated and vehicle-treated groups, comparing the mean lesion volumes using two-way ANOVA. Survival was determined from the animals used for the memory and neurologic motor studies, which were observed for 2 weeks following brain injury. Differences in survival between treatment groups were compared using Fisher's exact probability test. A  $p$  value of less than 0.05 was considered statistically significant.

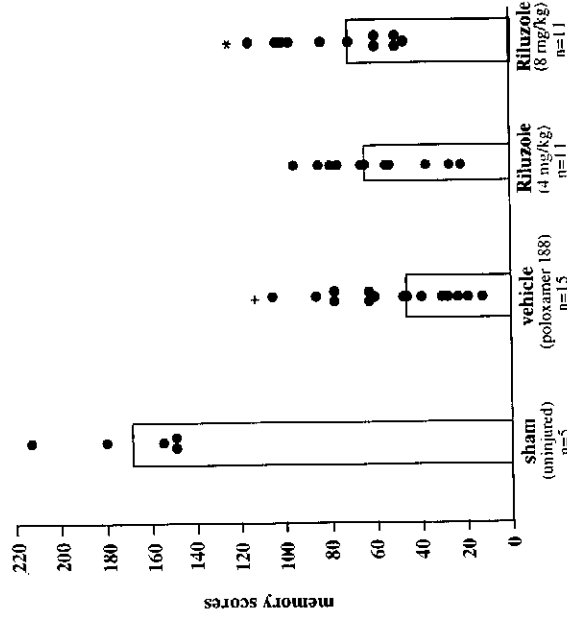
## RESULTS

FP brain injury induced a profound cognitive deficit (memory loss for the preinjury learned visuospatial task), as demonstrated by a greater than fourfold decrease in median memory scores of the injured vehicle-treated group compared with the uninjured vehicle-treated group ( $p < 0.001$ ) (Fig. 2). This memory loss was not affected by postinjury administration of low-dose Riluzole (4 mg/kg) (Fig. 2). However, administration of the higher dose of Riluzole (8 mg/kg) significantly improved post-traumatic cognitive function when compared with vehicle-treated brain-injured animals (median memory scores, 72 versus 47 for vehicle-treated animals,  $p < 0.05$ ) (Fig. 2). Injured animals treated with vehicle showed little platform-seeking behavior, whereas animals receiving Riluzole following brain trauma visibly retained memory of the location of the platform site. Following brain injury, no differences were observed between injured and uninjured animals with respect to swim speed or swim distance in the MWM, suggesting that posttraumatic motor deficits did not impair the ability of the brain-injured animals to perform this cognitive test.

Forty-eight hours after FP brain injury, significant regional edema was observed in the injured parietal cortex (site of maximal injury,  $p < 0.05$ ), left (ipsilateral) hippocampus ( $p < 0.05$ ), and left thalamus ( $p < 0.05$ ) when compared with uninjured (sham) control brain regions

## RILUZOLE: 42 hours postinjury

(15min i.v., 6h s.c., 24h s.c.)



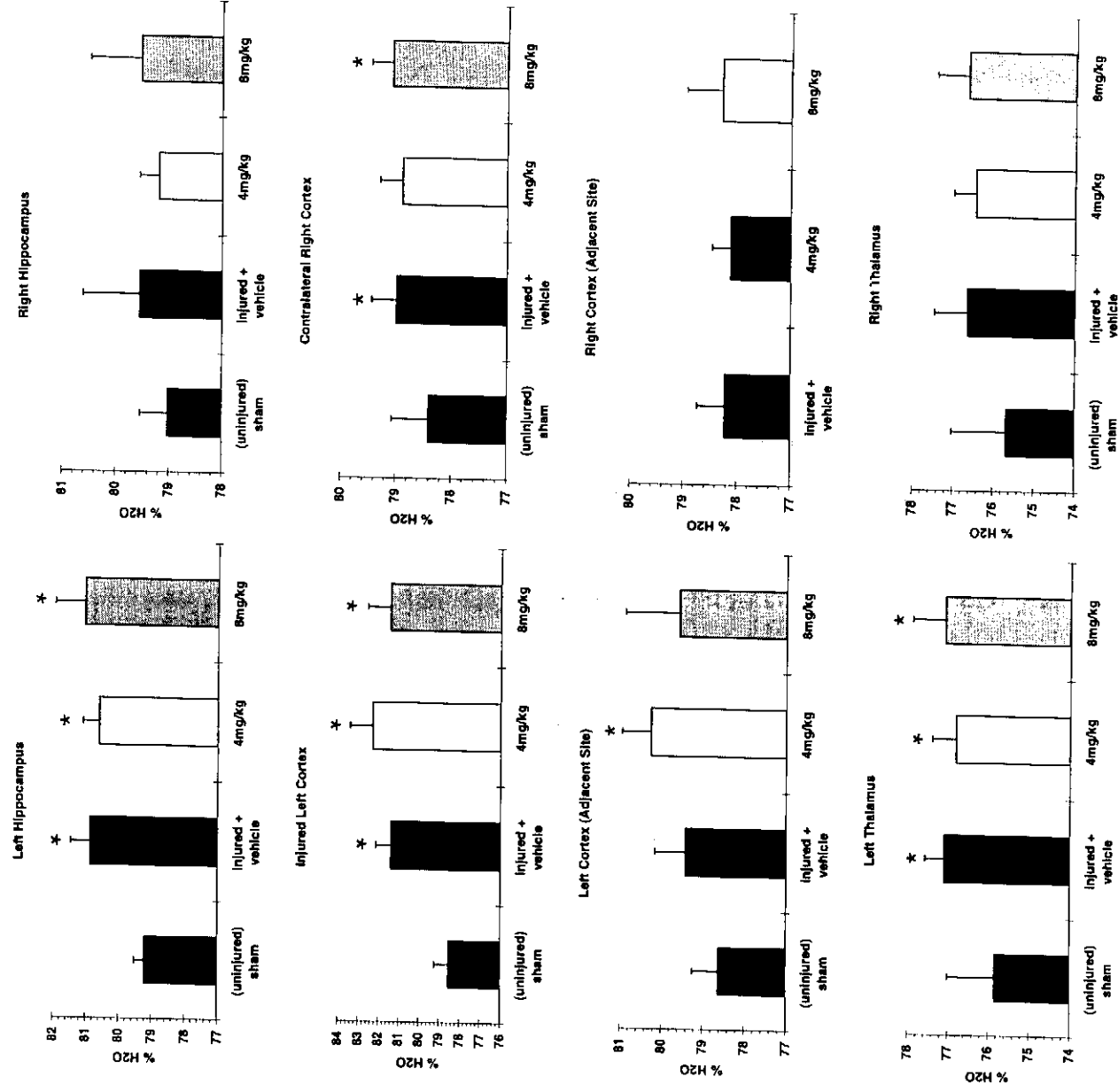
\* injured vehicle vs. injured drug (2,3,2.5 atm),  $p < 0.05$

+ injured vehicle vs. sham,  $p < 0.001$

**FIG. 2.** Memory scores from sham (uninjured) animals ( $n = 5$ ) and animals subjected to fluid-percussion brain injury and treatment with either vehicle ( $n = 15$ ), Riluzole (4 mg/kg,  $n = 11$ ), or Riluzole (8 mg/kg,  $n = 11$ ). \* $p < 0.05$  when compared with injured vehicle-treated animals. † $p < 0.001$  when compared with sham (uninjured controls).

(Fig. 3). Analysis of the right (contralateral) hippocampus, thalamus, and cortex contralateral to the maximal injury site revealed a significant increase in tissue water content only in the right contralateral cortex ( $p < 0.05$ ). Postinjury administration of Riluzole using the two dosing paradigms had no effect on the development of post-traumatic regional cerebral edema in any brain region examined (Fig. 3).

Fluid-percussion brain injury in vehicle-treated animals induced a significant neurologic motor function deficit when measured at 24 h, 48 h, 1 week, and 2 weeks following trauma ( $p < 0.01$ ) when compared with sham (uninjured) vehicle-treated animals (Fig. 4). Although significant effects on neurologic motor scores in Riluzole-treated animals (8 mg/kg  $\times$  3 doses) were not observed during the acute 24–48 h posttraumatic period, by 1 week postinjury, animals treated with Riluzole demonstrated a significant improvement in neurobehavioral deficits, as reflected by significant improvements in composite neurologic motor scores ( $p < 0.05$ ) when compared with vehicle-treated brain-injured animals



**FIG. 3.** Measurement of cerebral edema (% g water/g tissue) within selected brain regions in sham (uninjured) animals ( $n = 8$ ) and at 48 h following fluid-percussion brain injury and treatment with either vehicle ( $n = 11$ ), Riluzole (4 mg/kg  $\times$  3 doses,  $n = 11$ ), or Riluzole (8 mg/kg  $\times$  3 doses,  $n = 11$ ). \* $p < 0.05$  when compared with sham (uninjured) control values.

(Fig. 4). This improvement in posttraumatic motor function persisted in Riluzole-treated animals up to 2 weeks postinjury ( $p < 0.05$ ) (Fig. 4). When individual neurologic tests were analyzed separately, a significant improvement was observed in right-sided hindlimb flexion at 1 week ( $p < 0.02$ ) and right contraflexion scores at both 1 week ( $p < 0.005$ ) and 2 weeks ( $p < 0.02$ ) in Riluzole-treated animals when compared with vehicle-treated controls (Fig. 5).

Histologic evaluation of posttraumatic lesion volume at 48 h postinjury (Fig. 6) revealed that administration of Riluzole (8 mg/kg  $\times$  3 doses) had no significant effect on the size of the cortical lesion that develops following lateral FP brain injury in the rat. Brain-injured, vehicle-treated animals developed a significant cortical lesion ( $\bar{x} = 38 \pm 7 \text{ mm}^3$ ) by 48 h postinjury. Animals treated with Riluzole did not show any diminution of lesion size ( $\bar{x} = 41 \pm 12 \text{ mm}^3$ ,  $p = \text{N.S.}$ ).

## Riluzole : Composite Neuroscore

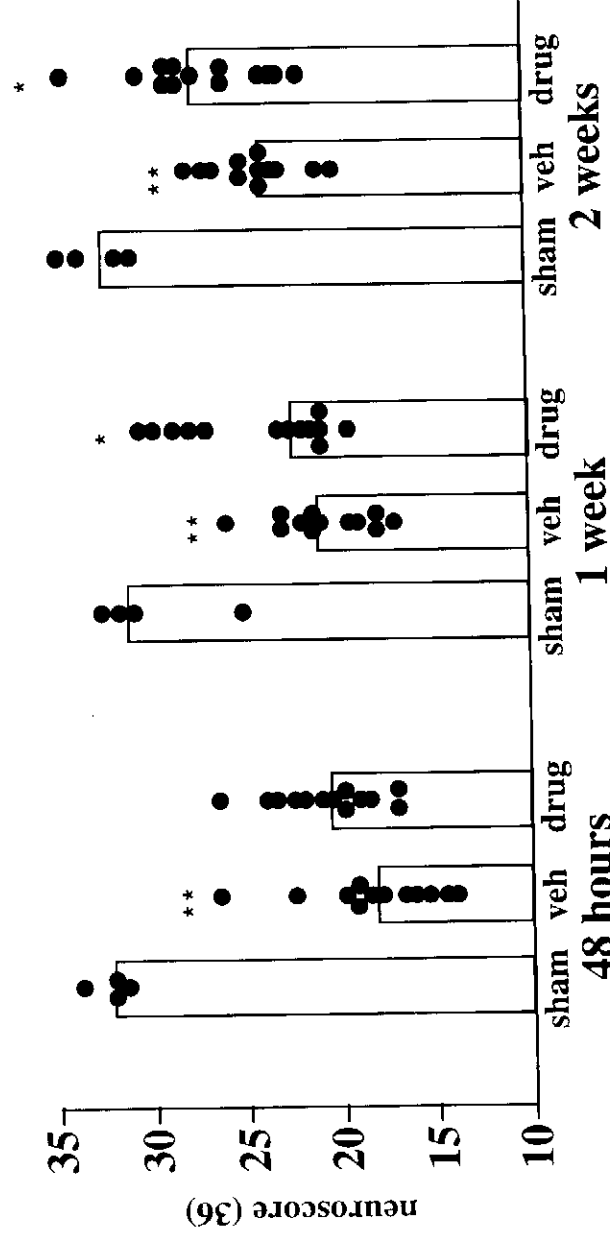


FIG. 4. Mean composite functional neurologic motor scores of sham (uninjured) animals ( $n = 4$ ) and animals subjected to fluid-percussion brain injury and treatment with either vehicle (veh) ( $n = 12$ ) or Riluzole (8 mg/kg  $\times$  3 doses,  $n = 13$ ). Scoring was evaluated at 48 h, 1 week, and 2 weeks following brain injury. Dots represent individual scores. \* $p < 0.05$  when compared with vehicle-treated, brain-injured animals. \*\* $p < 0.01$  when compared with sham (uninjured) animals.

A slight but nonsignificant decrease in temporalis muscle temperature (reflective of core brain temperature) of  $0.6^{\circ}\text{C} \pm 0.05$  (mean  $\pm$  SEM) was observed immediately following FP brain injury, which typically resolved by 30 min postinjury. Core body temperature did not change following brain injury (data not shown). Treatment with either dose of Riluzole (4 or 8 mg/kg) or vehicle had no effect on temporalis muscle or core body temperature following injury. Moreover, in sham (uninjured) animals, administration of Riluzole (4 or 8 mg/kg) had no effect on temporalis muscle or core body temperature (data not shown).

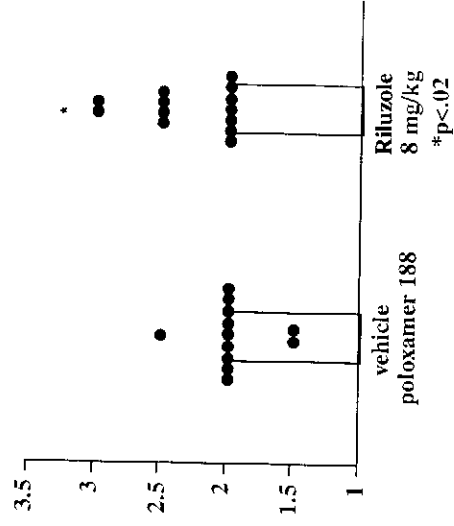
## DISCUSSION

In the present study, treatment with Riluzole beginning 15 min after FP injury was able to confer a significant degree of neuroprotection with respect to both cognitive and neurologic motor function, which lasted for weeks following brain injury and drug treatment. These behavioral improvements were not associated with a reduction of cerebral edema or posttraumatic lesion volume. Riluzole does have muscle relaxant prop-

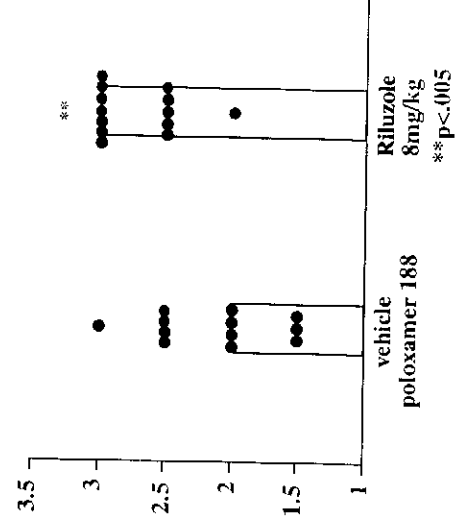
erties that persist for several hours postadministration, which may explain why Riluzole-treated animals did not show significant improvement on neurologic motor scores in the acute posttraumatic period. Sham (uninjured) animals treated with Riluzole do indeed show some sedation and muscle relaxation up to 4 h post-treatment (unpublished data), which may lower motor function scores. Although it has been reported that induction of brain or whole body hypothermia significantly reduced cell damage in rodent models of cerebral ischemia (Hayward et al., 1993; Zhang et al., 1993) and brain trauma (Clifton et al., 1991; Jiang et al., 1992; Lyeth et al., 1993; Taft et al., 1993; Dietrich et al., 1994b), our results appear to rule out any hypothermic effect, as Riluzole did not induce any alteration in core body or brain temperature.

Another possible mechanism related to the neuroprotective effects of Riluzole is the ability of this compound to reduce neuronal excitability and activity. With 2-deoxyglucose techniques, Riluzole has been shown to decrease neuronal activity in a dose-dependent fashion *in vivo* (Doble, 1996) and *in vitro* in cultured cerebellar granule cells and hippocampal slices (Miyazaki et al., 1989; Stutzmann et al., 1991; Doble et al., 1992). This

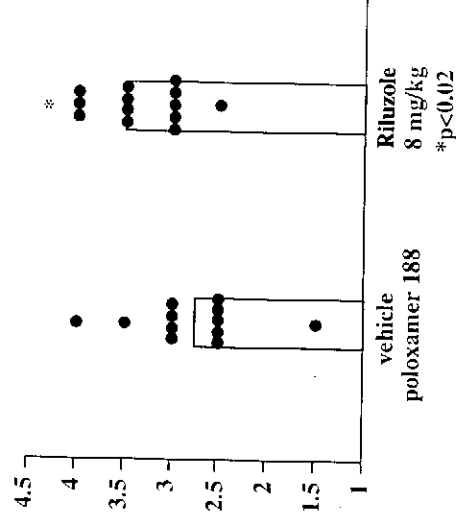
**Right Hindlimb Flexion: 1 week postinjury**



**Right Contraflexion: 1 week postinjury**



**Right Contraflexion: 2 weeks postinjury**



property has been attributed to the compound's ability to reduce cation flux into the neuron, thus protecting it from periods of pathologic depolarization and repolarization that accompany neuronal injury.

One possibly relevant mechanism of action is the ability of Riluzole to inactivate sodium channels (Benoit and Escande, 1991), with subsequent attenuation of glutamate release. Riluzole has been shown to stabilize the voltage-dependent Na<sup>+</sup> channel in its inactivated state and interacts with voltage-sensitive sodium channels by stabilizing the inactivated conformation of the channel protein (Benoit and Escande, 1991; Hebert et al., 1994). Riluzole and associated use-dependent sodium channel blockers BW1003C87 and 619C89 have been reported to attenuate the release of glutamate and aspartate in the striatum and cortex following focal cerebral ischemia (Lustig et al., 1992; Meldrum et al., 1992; Graham et al., 1993b). Preinjury treatment with 619C89 also has been shown to significantly reduce neuronal loss in CA1 and CA3 hippocampal pyramidal cell layers following lateral FP brain injury (Sun and Faden, 1995), and postinjury treatment with BW1003C87 and 619C89 can attenuate regional cerebral edema and improve neurobehavioral function, respectively, following FP brain injury (Voddi et al., 1995; Okiyama et al., 1995). Both 619C89 (Smith et al., 1989; Graham et al., 1993a; Leach et al., 1993) and Riluzole (Malgouris et al., 1989; Pratt et al., 1992) have been reported to protect against postischemic cell death in hippocampal CA1 regions and reduce hemispheric infarct volume. In addition to presynaptic blockade of glutamate release, Riluzole has been shown to inhibit NMDA-evoked acetylcholine release in rat striatal slices (Benavides et al., 1985) and NMDA-evoked calcium entry in cerebellar granule cells (Stutzmann and Doble, 1995). Although Riluzole significantly reduces glutamate and NMDA but not AMPA/kainate neurotoxicity in rat motoneuron cultures (Estevez et al., 1995), binding studies have not demonstrated a direct interaction of Riluzole with any of the known binding sites on the NMDA receptor complex or to AMPA, kainate, or metabotropic receptors (Doble and Perrier, 1989; Debono et al., 1993). Thus, the stabilization of the inactivated state of voltage-dependent sodium channels, with subsequent reduction of glutamate release, could also be responsible, in part, for the neuroprotective effects of Riluzole.

The hippocampus has been shown to be an integral modulator of spatial learning and memory (Olton et al.,

**FIG. 5.** Individual neurologic motor function scores of animals subjected to fluid-percussion brain injury and treatment with either vehicle ( $n = 12$ ) or Riluzole (8 mg/kg  $\times$  3 doses,  $n = 13$ ). Dots represent individual scores. \* $p < 0.02$  and \*\* $p < 0.005$  when compared with vehicle-treated injured animals.

## CORTICAL LESION VOLUME: Riluzole Vs. Vehicle

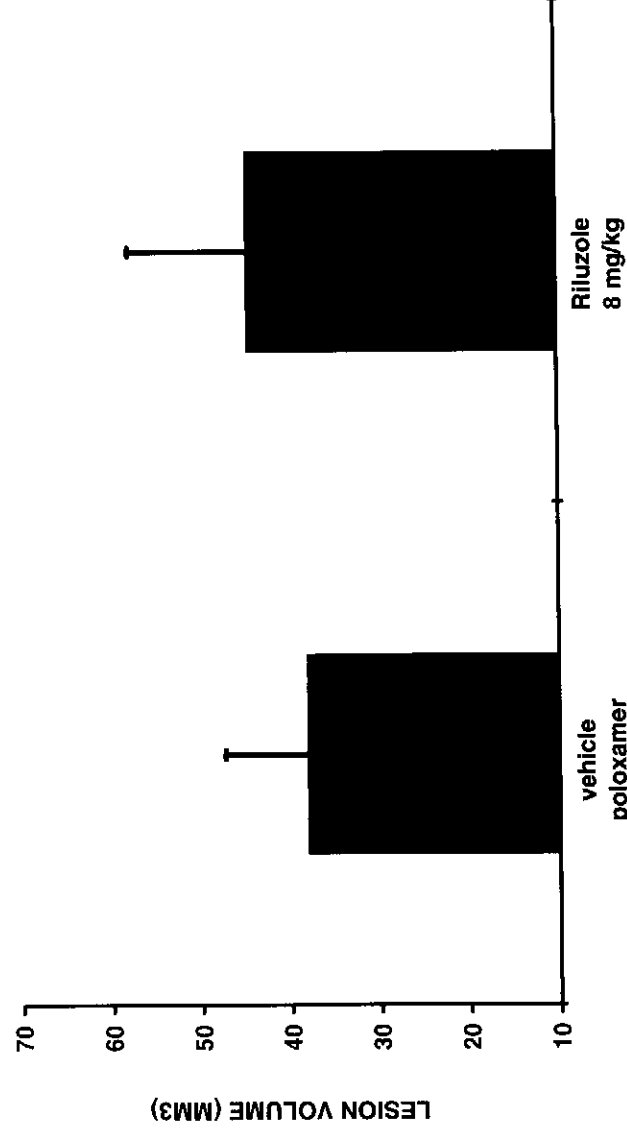


FIG. 6. Measurement of posttraumatic lesion volume ( $\text{mm}^3$ ) at 48 h postinjury in animals subjected to fluid-percussion brain injury and treated with either vehicle ( $n = 10$ ) or Riluzole (8 mg/kg  $\times$  3 doses,  $n = 10$ ), using TTC analysis.

1978; Morris et al., 1982), and both clinical and experimental studies have suggested that bilateral damage to the hippocampus may play a role in the development of posttraumatic memory disorders (Scoville and Milner, 1957; Zola-Morgan and Squire, 1986). The hippocampus contains the highest concentrations of NMDA receptors in the brain (Monaghan and Cotman, 1986), and a selective vulnerability of populations of neurons containing both NMDA and non-NMDA receptors, including hippocampal CA3 regions and the hilar cells of the dentate gyrus, has been demonstrated in rodent and primate models of experimental brain trauma (Cortez et al., 1989; Kotapka et al., 1991; Lowenstein et al., 1992; Hicks et al., 1993; Dietrich et al., 1994a). Moreover, a profound bilateral alteration in epileptic seizure threshold has been reported to occur following FP brain injury in the rat (Lowenstein et al., 1990). Memory dysfunction has been shown to be one of the most debilitating features of human TBI (Parkin, 1984; Levin, 1985), and both suppression of hippocampal long-term potentiation (LTP) and profound learning and memory impairment have been reported in a variety of models of experimental TBI (Miyazaki et al., 1989; Lyeth et al., 1990; Smith et al., 1991, 1994; Hamm et al., 1992, 1993a; Hicks et al., 1994). These posttraumatic cognitive deficits have been correlated with the excitotoxic-induced loss of neurons in hippocampal CA3 and hilar neurons of dentate gyrus

(Hicks et al., 1993). Administration of NMDA and non-NMDA receptor antagonists has been reported to improve cognitive function (Hayes et al., 1988; Faden et al., 1989; Shapira et al., 1990; Hamm et al., 1993b; Smith et al., 1993a,b; Hylton et al., 1995), attenuate loss of hippocampal microtubule-associated protein (MAP-2) (Hicks et al., 1995), and significantly reduce hippocampal cell loss (Hicks et al., 1994) following experimental brain injury in the rat. The improvement in cognitive function following postinjury administration of Riluzole in the present study may, therefore, be due to the ability of this compound to interfere with posttraumatic glutamate neurotoxicity, resulting in prevention of excitotoxic cell death in the hippocampus. Future studies will specifically address this possibility.

Previous studies have demonstrated significant improvements in posttraumatic motor dysfunction following treatment with NMDA receptor antagonists, such as the noncompetitive NMDA antagonists PCP (Hayes et al., 1988; Jenkins et al., 1988), magnesium chloride (McIntosh et al., 1989c), MK-801 (McIntosh et al., 1990; Shapira et al., 1990), dextromethorphan (Faden et al., 1989), HU-211 (Shohami et al., 1995), the competitive NMDA antagonist CPP (Faden et al., 1989), and the glycine-site antagonist indole-2-carboxylic acid (Smith et al., 1993b). However, compounds that interfere with excitotoxic damage are not uniformly active in TBI models. For example, MK-801 (or

dizocipine) appears to be inactive with respect to neurologic and histologic parameters when administered in the posttraumatic period to rats (McIntosh et al., 1989b; Toulmond et al., 1993) or mice (F. Wahl, personal communication). These inconsistencies may be related to the dose or timing of drug administration. As Riluzole does not possess any direct cerebrovascular effects and does not modify blood pressure (Doble, 1996), it may also possess some advantages over certain noncompetitive glutamate antagonists (e.g., MK-801), which, by virtue of their alpha-adrenergic effects, may be contraindicated in the clinic (Clineschmidt et al., 1982; Torregrosa et al., 1994). Moreover, although postsynaptic glutamate antagonism appears to be neuroprotective in various experimental models of brain trauma, potential psychomimetic side effects and associated neurotoxicity of several of these compounds may make them potentially unsuitable for clinical use (Olney et al., 1989; Sharp et al., 1991). An alternative to postsynaptic EAA antagonism may be to employ compounds, such as Riluzole, to inhibit the release of EAAs induced by traumatic CNS injury. This strategy has been successfully employed using the use-dependent sodium channel blocker/glutamate release inhibitors BW1003C87 and 619C89 in experimental models of cerebral ischemia (Meldrum et al., 1992; Graham et al., 1993a,b; Leach et al., 1993) and brain trauma (Okuyama et al., 1995; Sun and Faden, 1995).

Although modulation of postsynaptic EAA receptor function has been shown previously to attenuate post-traumatic regional cerebral edema in a variety of experimental models of brain injury (McIntosh et al., 1990; Shapira et al., 1990; Smith et al., 1993b; Okuyama et al., 1995), the administration of Riluzole in the present study did not affect regional cerebral edema. The reason for these differences is not clear at the present time. Riluzole was also observed to have no effect in reducing the extent of cortical lesion volume. However, little evidence exists to suggest that improvements in neurologic motor or cognitive deficits are dependent on reduction of cortical cell loss. Although Toulmond et al. (1993) reported that posttraumatic administration of Eliprodil, a polyamine-site antagonist, caused a marked reduction in the volume of cortical damage following lateral FP brain injury in the rat, neurologic motor function scoring was not performed in that study. However, a more recent study suggests that administration of Riluzole (8 mg/kg) is able to reduce cortical lesion volume by 41% (F. Wahl and J.-M. Stutzmann, unpublished observations). Future studies are planned using more detailed quantitative cell counting in vulnerable regions to assess more thoroughly the specific mechanisms underlying the neuroprotective properties of this compound.

Although it is possible that administration of Riluzole

at doses higher than 8 mg/kg  $\times$  3 would have produced more dramatic effects on behavioral outcome and may have positively affected edema and lesion volume, issues surrounding mortality and toxicity may be relevant. It is known that the acute lethal dose (LD<sub>50</sub>) via i.v. administration is 22 mg/kg in rats (J.-M. Stutzmann, personal communication). Moreover, preliminary studies conducted in our laboratory using a dose of 16 mg/kg i.v. and 2  $\times$  16 mg/kg s.c. produced an enhanced mortality in brain-injured rats. It remains possible, however, that administration of Riluzole at doses between 8 and 16 mg/kg may be increasingly beneficial in the treatment of brain trauma.

Finally, Riluzole has also been shown to possess indirect neurotrophic effects that may mediate, in part, the neuroprotective effects of this compound. Estevez et al. (1995) reported an increased number of neurons with increased length of neurites in cultures of motoneurons treated with Riluzole. These results suggest that Riluzole could act via direct or indirect neurotrophic effects, a property that may also participate in the protective activity of Riluzole in models of neurotrauma. Although the exact molecular targets of Riluzole are unknown, several distinct mechanisms of action are possible, including (1) inhibition of glutamate release, (2) stabilization of the inactivated state of voltage-dependent sodium channels, (3) noncompetitive blockade of EAA receptors, and (4) indirect neurotrophic effects of Riluzole. The precise mechanisms underlying the neuroprotective effects of Riluzole in models of brain trauma are currently under investigation.

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