Neurobehavioral protection by the neuronal calcium channel blocker Ziconotide in a model of traumatic diffuse brain injury in rats

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Object. Abnormal accumulation of intracellular calcium following traumatic brain injury (TBI) is thought to contribute to a cascade of cellular events that lead to neuropathological conditions. Therefore, the possibility that specific calcium channel antagonists might exert neuroprotective effects in TBI has been of interest. The focus of this study was to examine whether Ziconotide produces such neuroprotective effects.

Methods. The authors report that the acceleration–deceleration model of TBI developed by Marmarou, et al., induces a long-lasting deficit of neuromotor and behavioral function. The voltage-sensitive calcium channel blocker Ziconotide (also known as SNX-111 and CI-1009) exerts neuroprotective effects in this model of diffuse brain injury (DBI) in rats. The dose and time of injection of Ziconotide chosen for the present study was based on the authors’ previous biochemical studies of mitochondria. Rats were trained in a series of motor and memory tasks, following which they were subjected to DBI using the Marmarou, et al., model. At 3, 5, and 24 hours, all rats were injected with 2 mg/kg Ziconotide for a total cumulative dose of 6 mg/kg Ziconotide. Control brain-injured animals were injected with an equal volume of saline vehicle at each of these time points. The rats were tested for motor and cognitive performance at 1, 3, 7, 14, 21, 28, 35, and 42 days postinjury. Saline-treated rats displayed severe motor and cognitive deficits after DBI. Compared with saline-treated control animals, rats treated with Ziconotide displayed better motor performance during inclined plane, beam balance, and beam walk tests; improved memory while in the radial arm maze; and improved learning while in the Morris water maze.

Conclusions. These results demonstrated that the acceleration–deceleration model, which had been developed by Marmarou, et al., induces severe motor and cognitive deficits. We also demonstrated that Ziconotide exhibits substantial neuroprotective activity in this model of TBI. Improvement was observed in both motor and cognitive tasks, even though treatment was not initiated until 3 hours after injury. These findings support the development of neuronal N-type calcium channel antagonists as useful therapeutic agents in the treatment of TBI.

KEY WORDS • traumatic brain injury • calcium channel blocker • neurobehavioral deficit • neurobehavioral protection • rat

INTRACELLULAR calcium regulates many important cellular functions, including second messenger systems, gene expression, and neurotransmitter and hormone release. Therefore, it is not surprising that disruption of calcium homeostasis after brain injury can have devastating consequences for nervous system function. Excessive influx and intracellular accumulation of calcium after TBI have been linked to a cascade of neuropathological events that disrupt normal cell activity and lead to neuronal death and impaired brain function. As a result, there has been a major focus on the role of calcium in brain-injury mechanisms, as well as interest in the development of calcium channel blocking agents as potential neuroprotective agents after brain injury.

Entry of calcium into neurons is regulated in part by VSCCs. At least six types of VSCCs have been described including L, N, P, Q, R, and T. The L-, N-, and P-type VSCCs play major roles in the release of various neurotransmitters, including glutamate. Specific antagonists to the VSCCs have been developed. The L-type VSCC is selectively sensitive to blockade by 1,4-dihydropyridines such as nifedipine and nimodipine. The P-type VSCC is selectively blocked by various peptides that have been isolated from the venom of funnel-web spiders, such as ω-agatoxin-IVA, whereas the N-type VSCC is blocked by ω-conopeptides isolated from the venom of fish-hunting snails of the genus Conus. Earlier studies have examined the ability of the L-type VSCC blocker, nimodipine, to provide neuroprotection against brain injury caused by ischemia or trauma. Unfortunately, multiple clinical trials of nimodipine treatment in patients with brain injury have yielded largely negative results, although nimodipine does appear to provide some degree of neuroprotection in cases of traumatic subarachnoid hemorrhage.

The recently developed synthetic ω-conopeptide Ziconotide (also known as SNX-111 and CI-1009) has been

Abbreviations used in this paper: DBI = diffuse brain injury; TBI = traumatic brain injury; VSCC = voltage-sensitive calcium channel.
shown to block N-type VSCCs and to block release of various neurotransmitters. Recently it was observed that brain mitochondrial function is impaired after TBI, and to be antinociceptive in models of pain in animals. An additional and important finding concerning Ziconotide has been that it provides neuroprotection in a model of transient global forebrain ischemia, even when first administered as long as 24 hours after reperfusion. Ziconotide has been shown to protect neurons from degeneration in models of brain ischemia in animals, and to be antinociceptive in models of pain in animals.1,3,4,31,33

In the present study, therefore, we examined the ability of Ziconotide to provide neuroprotection against motor impairments and cognitive deficits in rats in a model of DBI. Diffuse brain injury was produced using the impact acceleration–deceleration model of brain injury described by Marmarou, et al. We have found that this model provides reliable motor and cognitive impairments and, therefore, provides a useful tool for examining the ability of experimental drugs, such as Ziconotide, to provide neurobehavioral protection and to improve behavioral outcome after brain injury. Initiation of Ziconotide treatment was delayed 3 hours after DBI, and a total dose of 6 mg/kg was used over a 24-hour period. These treatment parameters were chosen on the basis of our previous work demonstrating substantial neuroprotection of mitochondrial function after TBI by using 2 to 6 mg/kg of Ziconotide, with optimum effects produced by injections delayed 2 to 6 hours after brain injury.17,29,30

**Materials and Methods**

**Animal Preparation**

Twenty-four adult male Sprague–Dawley rats weighing 350 to 400 g each were used for the study. The rats were assigned to one of the following groups: an injured placebo-treated group (eight animals); an injured drug-treated group (nine rats); and a sham-injured control group used only for Morris water maze testing (seven animals). For all other tests no sham-injured control animals were necessary because preinjury data served as control data). The rats were housed in an environmentally controlled vivarium. All procedures were approved by the Wayne State University Animal Investigation Committee and conformed to the American Association for the Accreditation of Laboratory Animal Care guideline for the humane treatment of laboratory animals. The animals were allowed 1 week to adapt to the vivarium before the start of the experiments. An additional four animals died shortly after injury and they will not be further considered here. These animals died before randomization into treatment groups and, therefore, their exclusion did not affect group assignments or otherwise bias data.

**Behavioral Training**

Training was conducted in a dimly illuminated sound-shielded laboratory. The rats were placed on a restricted feeding schedule designed to maintain body weight at 90% of free-feeding weight. The animals were allowed 1 week to adapt to this schedule, and were then trained before brain injury in the following motor and cognitive tasks.

**Beam Balance Test.** The balance beam consisted of a 1.5 × 1.5–cm beam 1 m in length. The beam was painted white and suspended 1 m above the laboratory bench top. The rats were placed on the beam so that all four limbs were positioned on the top of the beam. The animals were required to balance on the beam for 60 seconds, after which time they were given a score for their time on the beam (maximum 60 seconds) as well as a score (1–6) for balance position (1, maintained balance and walked across the beam easily; 2, remained on the beam but balance was unsteady and the animal was hesitant about walking on the beam; 3, remained on the beam, but displayed obvious slipping and misplacement of forelimbs or hindlimbs; 4, maintained position by grasping the beam, but made no attempt to balance or move across the beam; 5, remained draped across the beam without grasping or moving across it, with or without falling; and 6, fell off the beam without attempting to balance or remain on it).

**Beam Walk Test.** The beam walk structure consisted of an illuminated start box and a darkened escape box that were connected by a 1.5–1.5–cm, 1-m-long walkway. The walkway had four 2-cm-high metal pins inserted off center in the walkway. This forced the animals to walk around the pins as they crossed the beam and served to increase task difficulty. The procedures and apparatus were similar to those described by Dixon, et al. Briefly, the animals were placed in the lighted start box and allowed 90 seconds to cross the walkway to the darkened escape box. A small food reward was also placed in the escape box (Froot Loops cereal; Kellogg Co., Battle Creek, MI) to facilitate performance. Animals underwent three trials per day and the amounts of time required to cross the beam were averaged across the three trials to yield a daily beam walk latency.

**Inclined Plane Test.** The rats were placed on a 20 × 30–cm platform, which was covered with rubber matting. The platform was inclined 60°. The rats were tested for their ability to remain on the platform (the inclined plane) for a maximum of 10 seconds.

**Radial Arm Maze.** The radial arm maze consisted of an elevated platform with eight 15 × 60–cm arms radiating outward from an octagonal center chamber. The slides of these arms were made of clear Plexiglas, and entry to the arms from the center was controlled by guillotine arms. The maze was baited with Froot Loops cereal pieces, which were placed in recessed cups at the ends of each arm of the maze. The rats were placed in the center of the maze, and doors to all eight arms were opened. The animals were allowed to enter and obtain food rewards located at the end of each of the eight arms. Training continued until the rats entered each arm only once and obtained all eight food rewards within a maximum of 5 minutes without reentry into a previously visited arm. Failure to enter an arm, reentry into a previously visited arm, or failure to obtain the food reward was recorded as an error and used to assess performance. The time required to complete the maze (that is, enter all eight arms) was also recorded, and a maximum of 300 seconds was allowed for each daily trial. The rats underwent one trial per day until they achieved a criterion of one or fewer errors on 2 consecutive days. They were then scheduled for brain-injury procedures on the following day, as later described. The radial arm maze was used as a test of spatial working memory and has been previously shown to be sensitive to hippocampal damage following lesions of the hippocampus or after TBI.

**Morris Water Maze.** The Morris water maze consists of a circular 1-m-diameter tank filled with opaque water (26°C). A 7.5-cm-diameter escape platform was hidden 1 dm below the surface of the water. The rats were placed in the water at the end of the tank, in one of four starting quadrants. The starting quadrant was randomly changed for each trial so that the rat was forced to learn the spatial location of the escape platform, rather than simply learn a swimming direction of response (that is, right as opposed to left). Rats were allowed 60 seconds to find and mount the escape platform. They were then removed from the maze and retested again 10 minutes later. Rats underwent one to four trials per day over 4 consecutive days. The time to find and to mount the escape platform was recorded, and all trials were videotaped. On the 5th training day, the position of the platform was moved and the animals were required to learn the new location of the platform over four consecutive 60-second trials. An additional control group of seven uninjured Sprague–Dawley rats matched for age and weight to the animals in the other groups were also tested in the Morris water maze, and their performances were used for comparisons with those of the Ziconotide- and saline-injected brain-injured animals.
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**Surgical Preparation and Brain Trauma Model**

The rats were placed in an inhalation chamber and anesthesia was initially induced by giving them a mixture of 5% halothane in a carrier gas mixture of 55% O2/44% N2O. The rats were then intubated and received mechanical ventilation (Harvard Apparatus, Holliston, MA) by using the O2/N2O gas mixture with halothane reduced to 1.5 to 2% during surgery and throughout the brain-injury procedures. A polyethylene cannula (Clay Adams PE-50; BD Biosciences, Franklin Lakes, NJ) was inserted into the left femoral vein. This catheter was used for Ziconotide (Neurex Corp., Menlo Park, CA) and saline-vehicle injections, which were administered after brain injury.

The DBI procedures were performed in the manner described by Marmarou, et al.18 The top of each animal’s head was shaved and a 1.5-cm-long scalp incision was made exposing the skull. The skull surface was cleared of connective tissue and dried. A 1-cm-diameter stainless-steel disk was cemented to the skull surface by using dental acrylic cement. The disk was positioned between the bregma and lambdoid skull landmarks. Three minutes after the disk was positioned, the anesthetized rats were secured to the top of a foam support. The disk mounted on the skull was positioned directly under a 2.5-m-tall, 3-cm-diameter clear Plexiglas tube. A 450-g brass weight inside the tube was allowed to precisely strike the disk cemented to the skull surface. Only a single strike was permitted to occur, and each rat was immediately removed from the brain-injury apparatus and immediately examined and scored for convulsion severity. In this model of DBI, rats exhibit a brief period of convulsions (10–40 seconds) immediately after injury. The duration and severity of the convulsions were recorded. Severity was rated on a scale of 0 to 3, with 0 indicating no convolution; 1, hindlimb twitching only; 2, mild hindlimb clonus; and 3, strong repetitive clonic motor convulsions. Ventilation and anesthesia were continued for an additional 5 minutes following brain injury while the scalp incision was sutured. Ventilation was then stopped, and the animals were examined every 3 minutes over the next 40 to 60 minutes for neurological signs of brain injury and return of reflex activity (such as eye blinking and righting reflex). These neurological measures were analyzed and used to ensure that the Ziconotide- and saline-treated groups received the same severity of brain injury.

**Postinjury Neurological Test Battery**

At 3-minute intervals, the rats were examined for hindlimb and forelimb flexion and abduction in response to calibrated pinch, eye blinking, and pinna reflexes in response to a light touch with a plastic probe, and the length of time required to regain the righting reflex. The length of time required to regain the righting reflex was used as an index of the duration of unconsciousness.

**Treatment With Ziconotide or Saline**

After they sustained DBI, the animals were coded and randomly assigned to either a Ziconotide-treated group (nine animals) or a saline-treated group (eight animals). The remaining animals (seven rats) were uninjured and were used in the Morris water maze as sham-injured controls for comparison with the injured groups. Investigators were blinded to each rat’s treatment condition. The rats were injected via the indwelling femoral vein catheter with either 0.2 ml of Ziconotide or saline vehicle. All rats were given a total of three injections of either Ziconotide or saline vehicle during the first 24 hours after brain injury. Specifically, Ziconotide-treated rats were injected with 2 mg/kg Ziconotide at 3, 5, and 24 hours postinjury, for a total cumulative dose during the 24 hours after injury of 6 mg/kg. Saline-treated control animals were injected with an equal volume of saline at the same three time points after injury. The dose and timing of drug injections were selected on the basis of our previous research, which demonstrated that Ziconotide was most effective in preserving mitochondrial function when given after a 2- to 6-hour delay and remained effective when given as long as 10 hours after brain injury.22,25 Similarly, Ziconotide has been reported to be effective in reducing ischemic brain injury, even when administered 24 hours after reperfusion.20 The Ziconotide solution was prepared immediately before use by placing the drug in sterile saline at a concentration of 2 mg/0.2 ml. Both saline vehicle and drug injections were given at a rate of 0.2 ml/minute.

**Retesting of Animal Behavior After Brain Injury**

The rats were retested for their ability to stay on the inclined plane, to maintain balance on the balance beam, to walk across the beam, and to perform in the radial arm maze accurately, by using the same procedures described earlier. Again, researchers were blinded to each animal’s treatment condition during retesting. Testing began 6 hours after injury and at least 3 hours after the last Ziconotide injection. Animals were retested in these tasks 3, 7, 14, 21, 28, 35, and 42 days later. After completion of testing in these tasks, the animals were then tested for spatial memory in the Morris water maze as described later. The water maze was used because it has been shown to be sensitive to various models of TBI, particularly those associated with damage to the hippocampus. It is also considered to be a test of spatial mapping ability, which has also been associated with hippocampal function.

**Statistical Analysis**

The performance measures collected during the postinjury neurological battery test and during each of the neurobehavioral testing procedures were analyzed using a repeated-measures analysis of variance followed by individual post hoc group comparisons when appropriate. The minimum level of statistical significance was set at a probability value less than 0.05.

**Results**

The rats were examined for approximately 60 minutes beginning immediately after brain injury, but before the scheduled administration of Ziconotide or saline treatments. Four rats died during this initial observation period. Duration and severity of convulsions, as well as the return of reflex activity (hindlimb movement, eye blinking, and ear twitch) and the righting reflex were measured. These data were analyzed at the end of the experiment to ensure that the animals scheduled to have received treatment with Ziconotide or saline initially exhibited the same level of injury severity. The data are presented in Table 1. As shown in the table, there was no significant difference in convulsion severity or duration, or in the time for return of reflex activity between groups before experimental drug treatment.

**Behavioral Assessment After Brain Injury**

**Inclined Plane Test.** The results of the inclined plane test are shown in Fig. 1. All animals showed an impaired ability to perform this task after brain injury. However, Zi-
Ziconotide-treated animals showed significantly better performances than saline-treated control animals beginning on the 1st day of testing. All animals eventually returned to the performance criterion for this task (that is, remaining on the inclined plane for a minimum of 10 seconds). Statistical analysis indicated a significant group difference (F{sub 1,12} = 6.04, p < 0.05), as well as a significant group-by-test-day interaction (F{sub 7,84} = 2.62, p < 0.05). Individual group comparisons across test days indicated that Ziconotide-treated animals remained on the apparatus significantly longer than control animals on Day 1 (p < 0.05), with marginal differences on test Days 3 (p < 0.07) and 7 (p < 0.09).

**Beam Balance Test.** Performance during the beam balance test is shown in Fig. 2 upper (duration) and center (balance score). As shown in Fig. 2 upper, all animals were able to remain on the balance beam for the full 60 seconds before they sustained brain injury. After injury, however, all animals were impaired in this task. The greatest degree of impairment was observed 1 day after injury, with incomplete recovery by Day 42. Animals injected with Ziconotide were significantly less impaired in this task than vehicle-treated control animals, and recovered by Day 42 to nearly preinjury performance. This difference between groups was statistically significant (F{sub 1,10} = 8.89, p < 0.05). Test day was statistically significant (F{sub 9,90} = 10, p < 0.01), but not group-by-test-day interaction. Similarly, the beam balance scores shown in Fig. 2 center demonstrate that all groups were impaired in their balance after injury. The scores indicate the degree to which rats were able to assume a normal balance posture on the balance beam. Although animals were able to remain on the balance beam for longer periods of time as recovery progressed (Fig. 2 upper), the balance score remained high, indicating an enduring motor deficit. Again, Ziconotide-treated animals performed this task better during repeated testing than saline-treated control animals. Statistical analysis across postinjury test days indicated a significant group difference (F{sub 1,12} = 4.6, p < 0.05) and a significant test day difference (F{sub 7,84} = 5.8, p < 0.01), with no significant interaction.

**Beam Walk Test.** Performance on the beam walk test is shown in Fig. 2 lower. Significant impairment was ob-
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Ziconotide-treated animals exhibited faster recovery than saline-treated control animals, approaching their preinjury performance level by test Day 42. Statistical analysis showed a significant group difference ($F_{1,12} = 8.65, p < 0.05$), as well as a significant group-by-test-day interaction ($F_{8,96} = 2.04, p < 0.05$). Individual group comparisons across test days indicated that groups differed significantly on test Days 35 and 42 ($p < 0.05$).

**Radial Arm Maze.** Two measures were used for evaluation of radial arm maze performance: mean time to complete the maze (Fig. 3 upper) and the mean number of errors per day (Fig. 3 lower). As shown in Fig. 3 upper, all rats completed the maze within 100 to 120 seconds before brain injury. In contrast, on postinjury test Days 1, 3, and 7, most animals in both treatment groups failed to complete the maze within 5 minutes. Beginning on Day 14, animals in the Ziconotide-treated group were faster in completing the maze than animals in the saline-treated control group, and this group difference was statistically significant ($F_{1,13} = 7.7, p < 0.02$). Individual group comparisons showed that Ziconotide-treated animals performed significantly faster than saline-treated animals on test Days 21, 28, and 35 ($p < 0.05$).

An analysis of errors made while animals were in the radial arm maze is shown in Fig. 3 lower. As shown in this figure, animals treated with Ziconotide made fewer errors than saline-treated control animals by test Day 14. However, this difference only reached marginal significance on test Day 14 ($p < 0.07$).

**Morris Water Maze.** Animals were tested for water maze performance 50 days after suffering DBI. Individual trials given on each day of testing were pooled for statistical analysis. The results are shown in Fig. 4. Statistical analysis demonstrated a significant group effect ($F_{2,21} = 7.92, p < 0.01$) and a significant effect of days ($F_{3,63} = 17.6, p < 0.01$). Individual group comparisons demonstrate that the sham-injured (uninjured) control animals and the Ziconotide-treated animals escaped significantly faster than the saline-treated animals ($p < 0.05$). On test Day 2 the uninjured control animals performed significantly better than saline-treated animals ($p < 0.01$), with the Ziconotide-treated group not differing from either group. On test Days 3 and 4 Ziconotide-treated animals and uninjured control animals performed significantly better than saline-treated animals ($p < 0.05$). There was no statistically significant difference among groups after the escape platform had been moved to a new location on test Day 5.

**Discussion**

To our knowledge, the present results are the first to demonstrate that the Marmarou, et al., 18 model of DBI results in enduring motor and cognitive deficits. Motor deficits were observed across a variety of tasks, including tests of balance (beam balance and beam walk), strength (inclined plane), and locomotion (radial arm maze). In addition, brain-injured animals showed poorer performance during tests of learning and memory (radial arm maze and
Morris water maze). Marmarou, et al., provided evidence for diffuse axonal damage in rats in this model, and Folkerts, et al., have also reported disruption in microtubular structure and fragmentation of dendrites. Thus the present results demonstrate that diffuse brain damage in this impact–acceleration procedure can also provide a useful model of neurobehavioral deficits. The fact that such deficits were observed across a variety of motor and cognitive tasks is likely to reflect the diffuse nature of the injury and the wide involvement of the nervous system at many levels, 8,18.

In the placebo-treated group at 6 weeks, scores on the inclined plane test and the radial maze were the only scores to have returned to baseline: all other test results still showed statistically significant differences in comparison with preinjury control data. In both these tests, the Ziconotide-treated animals returned to baseline or close to it 2 weeks earlier than the saline-treated group. Although 2 weeks may not appear important, one should take into consideration the approximately 100-week lifespan of the rats and then infer a 2%-lifespan or, in humans, an 18-month advantage for drug-treated groups. This would most certainly be clinically significant.

In the other five tests return to baseline had not taken place as of 6 weeks posttreatment, and in all of these parameters the Ziconotide-treated group clearly performed better over the whole course of the 6 weeks. Even 6 weeks after brain injury, Ziconotide-treated animals displayed less impairment in learning the Morris water maze compared with saline-treated control animals. The Morris water maze was used as a test of new learning after brain injury, compared with the other motor (beam walk, beam balance, and inclined plane) and learning (radial arm maze) tasks for which animals had been trained prior to injury. Therefore, the Morris water maze results indicate that treatment with Ziconotide after brain injury improved new learning skills, in addition to facilitating reacquisition of previously learned motor and cognitive skills.

The rats were randomly assigned to drug or placebo treatment after injury to avoid possible bias in varying the severity of the injury. Statistical analysis of convulsion severity and reflex activity measured immediately after brain injury, but before drug treatment, indicated that both treatment groups were initially injured to the same degree of severity. Therefore, it is unlikely that the present results are due to any systematic initial differences in the degree of brain injury severity between groups.

It is noteworthy that neuroprotective effects of Ziconotide were found, even though the initiation of Ziconotide treatment was delayed by 3 hours after injury. These findings are consistent with those of other reports that Ziconotide provides substantial protection against ischemia-induced brain injury when given as long as 24 hours after brain insult. 3,10 The present results are also consistent with findings of our earlier study that showed that brain mitochondrial function was best preserved after TBI when Ziconotide treatment was delayed by 2 to 6 hours after focal cortical injury. 3,23 This highly desirable property of delayed effectiveness could theoretically provide an important therapeutic window for treatment of clinical brain injury. Cellular damage following TBI undoubtedly involves more than just mitochondrial dysfunction. Therefore, it is perhaps not surprising that behavioral outcome representing the integrated function of the nervous system showed somewhat less complete neuroprotection by Ziconotide than that observed for mitochondria. 10 Nevertheless, protection against the motor and cognitive effects of brain injury by Ziconotide were substantial, particularly in this model of DBI, indicating the importance of N-type VSCCs in TBI.

The mechanisms by which Ziconotide produces neuroprotection are not currently understood, but are thought to be due to its ability to block newly discovered neuronal N-type calcium channels. Such VSCCs are known to play an important role in regulating release of neurotransmitters, including glutamate. If glutamate contributes to secondary brain injury, delayed injections of Ziconotide may limit such damage by preventing excessive glutamate release. 14,23 Alternatively, calcium influx through VSCCs after brain trauma may directly activate secondary mechanisms of injury. Disruption of calcium homeostasis after brain injury impairs brain mitochondrial function in both animals and humans. 31,33,36 In addition, calcium is known to activate calcium-dependent proteolytic enzymes such as the neutral protease calpain II, which has already been implicated as a mediator of secondary brain injury. Calcium may also participate in the generation of free radicals through stimulation of nitric oxide production or activation of phospholipase A_2. Thus, calcium probably contributes to secondary brain injury through a variety of mechanisms. In addition, intracellular calcium levels can increase over 24 to 48 hours after brain injury. 7 When blockade of calcium-related injury mechanisms is strictly limited to the period of time immediately following trauma, the continued effects of calcium accumulation may still be sufficient to produce substantial secondary brain injury. Under these conditions, delayed administration of calcium channel blockers may actually be more effective, particularly if drug delivery is extended over several hours after brain injury, as was done in the present study. Administration of VSCC blockers that takes place either too soon or too late, after damage has already occurred, may, therefore, be much less effective. Interference with these calcium-mediated mechanisms by blockade of the N-type VSCC could explain the neuroprotective actions of Ziconotide. 5,23,26,32,33

The present study raises several important questions concerning the role of calcium in mediating secondary brain injury, as well as the potential for calcium channel blockers to prevent injury or otherwise facilitate recovery. For example, the precise dose–response profile and the maximally effective time window for neurobehavioral protection provided by Ziconotide need to be established. Hayes (RL Hayes, personal communication, 1997) administered Ziconotide by continuous infusion over 24 hours in doses ranging from 1 to 3 mg/kg, beginning immediately after controlled cortical impact injury in rats. In that study, Ziconotide failed to produce measurable behavioral neuroprotection. The neuroprotection observed in the present study may, therefore, be the result of either the higher dose of Ziconotide that was used or its delayed administration, which could more effectively influence a critical period for calcium effects on brain injury. This is supported by our earlier findings that delayed injections of higher doses (for example, 4–6 mg/kg) of Ziconotide preserved mitochondrial function better than immediate pre-
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or postinjury administration, and/or lower doses.\textsuperscript{2,3} Alternatively, Ziconotide treatment may be more effective in diffuse, rather than focal brain injury, as was used by Hayes (RL Hayes, personal communication, 1997). These questions are presently under investigation in our laboratory. Second, the mechanisms of action of Ziconotide (for example, interference with neurotransmitter release, free radical production, or intracellular enzyme activity) need to be more carefully studied. Finally, it will be important to establish the primacy of specific N-channel blockade with Ziconotide for neuroprotection compared with blockade of other calcium channels (for example, P or Q).

Conclusions

The model of experimental head injury developed by Marmarou, et al.,\textsuperscript{18} induces a wide and long-lasting deficit of neuromotor and behavioral function. To our knowledge, Ziconotide is the first drug to exert a significant effect on all aspects of neuropsychological deficits after severe experimental TBI, although the dosage and timing of administration were derived from easily reproducible biochemical studies.\textsuperscript{2,3} The effectiveness and extended time window for treatment when using this compound provide an ideal opportunity to test this drug in the real world of clinical practice.\textsuperscript{32,33}

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