Delayed, Post-Injury Treatment with Aniracetam Improves Cognitive Performance after Traumatic Brain Injury in Rats

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ABSTRACT

Chronic cognitive impairment is an enduring aspect of traumatic brain injury (TBI) in both humans and animals. Treating cognitive impairment in the post-traumatic stages of injury often involves the delivery of pharmacologic agents aimed at specific neurotransmitter systems. The current investigation examined the effects of the nootropoic drug aniracetam on cognitive recovery following TBI in rats. Three experiments were performed to determine (1) the optimal dose of aniracetam for treating cognitive impairment, (2) the effect of delaying drug treatment for a period of days following TBI, and (3) the effect of terminating drug treatment before cognitive assessment. In experiment 1, rats were administered moderate fluid percussion injury and treated with vehicle, 25, or 50 mg/kg aniracetam for 15 days. Both doses of aniracetam effectively reduced injury-induced deficits in the Morris water maze (MWM) as measured on postinjury days 11–15. In experiment 2, injured rats were treated with 50 mg/kg aniracetam or vehicle beginning on day 11 postinjury and continuing for 15 days. MWM performance, assessed on days 26–30, indicates that aniracetam-treated animals performed as well as sham-injured controls. In experiment 3, animals were injured and treated with aniracetam for 15 days. Drug treatment was terminated during MWM testing on postinjury days 16–20. In this experiment, aniracetam-treated rats did not perform better than vehicle-treated rats. The results of these experiments indicate that aniracetam is an effective treatment for cognitive impairment induced by TBI, even when treatment is delayed for a period of days following injury.

Key words: aniracetam; brain injury; cognitive recovery; Morris water maze; rat; traumatic brain injury

INTRODUCTION

As understanding of the pathological processes of traumatic brain injury (TBI) has advanced, there has been great hope for acute interventions that would reduce the morbidity and mortality of TBI. Thus, research efforts have largely been directed towards pharmacological agents that alter the immediate neurochemical cascades triggered by TBI. Research in animal models of TBI has had great success, and a number of pharmacotherapies have been shown to reduce behavioral and histological complications (McIntosh et al., 1998). However, clinical investigations of acute, post-injury agents have been disappointing (Bullock et al., 1999; Maas et al., 1999).

Compared to the acute effects of TBI, the mechanisms that mediate chronic disability after TBI have been less intensively investigated and are not fully understood.
However, as early as 1905, Von Monakow (1969) hypothesized that the CNS enters a state of “functional depression” following insult. This theory of diachisis has been revised by Feeney (1991) as “remote functional depression” (RFD). The theory of RFD posits that the behavioral deficits observed after neurological insult are due, in part, to a functional depression of normal neuronal activity. Importantly, such behavioral deficits may occur in the absence of mass lesion, especially in midline fluid-percussion model, where histological damage is not common (Lyeth et al., 1990). According to this model, delayed post-injury therapeutic agents should be directed at increasing neuronal activity in the more chronic post-injury phase in order to return neuronal function into the normal range. Such interventions would be predicted to improve recovery from TBI even if initiated in the later stages of injury (Feeney, 1997).

Experimental studies have supported the predictions of RFD theory. For example, NE levels rise acutely after experimental TBI (Levin et al., 1995) but are decreased in the chronic post-injury phase (Dunn-Meynell et al., 1994; Pasad et al., 1994). Likewise, dopaminergic (DA) neurotransmission undergoes a similar reversal in activity between the acute (Huger et al., 1979) and chronic (McIntosh et al., 1994) phases. Many features of cholinergic receptor function are also depressed chronically after TBI (Dixon et al., 1994, 1996, 1997; Leonard et al., 1994). Thus, pharmacological treatments administered during the chronic post-injury phases should increase activity in these neuronal systems. Direct intraventricular infusion of NE facilitates recovery following cortical ablation injury (Boyeson and Fenney, 1990), as does administration of the NE precursor L-DOPS (Kikuchi et al., 2000) and the tricyclic antidepressant desipramine (Boyeson et al., 1993). As with NE, pharmacotherapy to increase DA levels in the subacute phase accelerates recovery from neurobehavioral deficits of hippocampal lesions and the somatosensory neglect of hypothalamic lesions (Feeney and Sutton, 1989). Other DA agonists, including amantadine (Dixon et al., 1999) and bromocriptine (Kline et al., 2002), have been shown to improve cognitive recovery when administered chronically following TBI. However, unlike NE, administration of DA agonists does not affect motor recovery in rat models of TBI (Feeney et al., 1993). As predicted by the RFD hypothesis of depressed neuronal function after TBI, a number of positive modulators of cholinergic function have been found to improve behavioral function after injury (Dixon et al., 1997; Pike and Hamm, 1995, 1997).

Since glutamate is the primary excitatory neurotransmitter in the brain, an examination of chronic, trauma-induced changes in this key neurotransmitter is critical to understanding the mechanisms that contribute to chronic neuronal dysfunction and the enduring cognitive deficits produced by TBI. The current experiment examined the AMPA subtype of the glutamate receptor. AMPA-glutamate receptors are ligand-gated, fast-acting channels that contribute to the neuron’s ability to fire rapidly by allowing Na⁺ to enter the cell, decreasing the time necessary to reach threshold values for an action potential. If TBI results in impaired AMPA receptor function, a reduction in neuronal activity would be predicted. Thus, a reduction in AMPA-glutamate receptors may contribute to the diachisis/RFD and result in chronic post-injury functional deficits.

Aniracetam, the treatment tested in the present experiment, has modulatory actions through the allosteric potentiation of AMPA-specific glutamate receptors. The consequences of this drug are a reduction of glutamate receptor desensitization and potentiation of metabotropic glutamate activity (Pizzi et al., 1993). The net effect of these aniracetam-induced alterations is an increase in neuronal activity. If a reduced level of neuronal activity is a long-term consequence of TBI, then augmenting neuronal activity with aniracetam should improve recovery from TBI. In addition to testing the efficacy of aniracetam in reducing cognitive impairments produced by TBI, this research examined the issues of dosage and time of treatment as they influence the drug’s efficacy in improving cognitive function after TBI.

METHODS

Subjects

Adult (3-month-old) male Sprague-Dawley rats (Hilltop Lab Animals, Inc., Scottsdale, PA) weighing 300–350 g were used in all experiments. Animals were housed individually (at 20–22°C with lights on from 06:00 to 18:00) with free access to food and water. All procedures followed the guidelines established in the Guide for the Care and Use of Laboratory Animals (U.S. Department of Health and Human Services) and were approved by our Institutional Animal Care and Use Committee.

Fluid Percussion Apparatus

The fluid percussion device used to produce experimental brain injury was identical to that used previously on rodents and is described in greater detail elsewhere (Dixon et al., 1987). Briefly, the device consisted of a Plexiglas cylinder reservoir 60 cm long and 4.5 cm in diameter. Fitted at the end of the metal housing is a 5-mm tube with a 2.6-mm inner diameter that terminated with a male Leur-Loc fitting. This fitting was connected to a female Leur-Loc fitting that had been implanted over the
exposed dura of the rat. The entire system was filled with distilled water. Injury is produced by a metal pendulum that strikes the piston of the injury device, injecting a volume of water into the closed cranial cavity and producing a brief displacement and deformation of brain tissue. The extracranial pressure pulse is expressed in atmospheres (atm).

**Surgical Preparation and Injury**

All animals were surgically prepared under 2% isoflurane anesthesia in a mixture of 70% N\textsubscript{2}O and 30% O\textsubscript{2}, 24 h before fluid percussion injury or sham injury. A 4.8-mm hole was trephined into the skull over the sagittal suture, midway between bregma and lambda. Two stainless steel screws were placed 1 mm rostral to bregma and 1 mm caudal to lambda. A modified Leur-Loc syringe hub with a 2.6-mm inside diameter was placed over the exposed dura and bonded in place with cyanoacrylate adhesive.

Twenty-four hours after the surgical preparation, rats were anesthetized with 4% isoflurane in a mixture of 70% N\textsubscript{2}O and 30% O\textsubscript{2} and connected to the injury device. Animals in the injured condition were then injured at 2.1–2.2 atm. This level of injury produces a moderate severity of injury that is associated with long-lasting cognitive deficits. Rats assigned to the sham-injury condition were anesthetized and connected to the injury device but not injured.

**Morris Water Maze**

The Morris water maze (MWM) procedure employs a 180-cm-diameter and 60-cm-high metal pool painted white and filled with water to a depth of 28 cm. The water temperature was maintained at 23–26°C. A 10-cm platform, located 2 cm below the water’s surface, was used as the hidden goal platform. The pool was located in a 2.5 × 2.5 m room with numerous extramaze cues (e.g., windows, pipes, book case) that remained constant throughout the experiment.

Rats were given four trials per day for 5 consecutive days. On each trial, rats were placed in the pool by hand at one of the four start locations (south, west, north, and east) facing the wall. Each animal started a trial once from all possible start locations on each day. The order of starting locations was randomized each day. The goal platform was positioned 45 cm from the outside wall and was placed in the center of the northeast quadrant. Rats were given a maximum of 120 sec to find the hidden platform. If the rat failed to find the platform after 120 sec, it was placed on the platform by the experimenter. All rats were allowed to remain on the platform for 30 sec before being placed in a heated incubator between trials.

There was a 4-min intertrial interval. The animal’s movements within the maze were recorded and analyzed with a video tracking system (San Diego Instruments, Polytrack 4). This tracking equipment allows analysis of both latency to reach the goal platform and swim speed. Latency to find the platform was the primary dependent variable for the assessment of cognitive performance. Swim speed was calculated to ensure that cognitive performance was not confounded by motor deficits that could slow swimming performance.

**EXPERIMENTS**

**Experiment 1: Aniracetam Treatment—Determination of Optimum Dose**

Experiment 1 was conducted to determine the most effective dose of aniracetam for reducing cognitive deficits after brain injury. Aniracetam has been found to be nootropic (cognitive enhancing) in a number of models of cognitive dysfunction. For example, it has been shown to improve fear conditioning (Lu and Wehner, 1997), object recognition (Lebrun et al., 2000), and spatial learning after cortical lesions (Zajaczkowski and Danysz, 1997). The doses selected for testing (25 and 50 mg/kg) were based on this research.

**Procedure.** Aniracetam (Tocris, Ellisville, MO) was suspended in 0.25% carboxymethylcellulose (CMC) solution containing a few drops of Tween 80 and was administered by oral gavage at a volume of 5 mL/kg. Beginning 24 h after midline fluid-percussion injury, either 25 \((n = 9)\) 50 mg/kg \((n = 9)\) of aniracetam, or vehicle \((0.25\%\text{ CMC and Tween 80, }n = 10)\) was administered daily for 15 days. An additional 10 rats were sham injured and untreated. On days 11–15 after TBI, rats were tested in the MWM, and the latency to reach the goal platform and swim speed were recorded.

**Results.** The mean latency to reach the goal platform is presented in Figure 1. These data were analyzed by a 4 (group) × 5 (day) split-plot analysis of variance (ANOVA). The ANOVA indicated a significant main effect of group \((F_{1,33} = 5.29, p < 0.004)\). To determine specific group differences, the Duncan Multiple Range test was used. The results of this test indicate that compared to injured-vehicle-treated rats, injured rats treated with 25 and 50 mg/kg of aniracetam demonstrated significantly improved MWM performance \((p < 0.05)\). In fact, the MWM performance of injured-aniracetam-treated rats (both 25 and 50 mg/kg) did not differ significantly from that of sham-injured rats. Analysis of swim speed found no group differences (data not shown).
Experiment 2: Aniracetam Treatment—Delayed-Chronic Administration

The purpose of this experiment was to investigate the optimum therapeutic strategy by examining the temporal therapeutic window for the posttraumatic pharmacological treatment of injury-induced cognitive impairment with aniracetam. In this experiment the efficacy of aniracetam in attenuating cognitive deficits was tested when chronic administration of the compound is delayed for 11 days following injury.

Procedure. Animals were treated with the optimal dose of aniracetam (50 mg/kg, oral, \( n = 8 \)) or vehicle (\( n = 10 \)) beginning on day 11 after injury. Daily oral injections were continued for the remainder of the experiment. Cognitive performance was assessed after 15 days of drug treatment on days 26–30 post-injury in the MWM. For comparison purposes, the MWM data from the sham-injured rats from Experiment 1 were included.

Results. The mean latency to reach the goal platform (Fig. 2) was analyzed by a 3 (group) × 5 (day) split-plot ANOVA. The ANOVA indicated there was a main effect of group (\( F_{2,25} = 4.96, p < 0.015 \)). Duncan’s Multiple Range test indicates that compared to injured-vehicle-treated rats, animals treated with 50 mg/kg of aniracetam beginning 11 days after TBI performed significantly better in the MWM compared to injured-vehicle-treated animals (\( p < 0.05 \)). In fact, the MWM performance of rats receiving delayed aniracetam treatment did not differ significantly from sham-injured rats. Analysis of swim speed found no group differences (data not shown).
Experiment 3: Aniracetam Treatment—Termination of Chronic Administration

This experiment tested whether the continued administration of aniracetam was necessary for the improvement in cognitive performance after TBI. In other words, does the chronic administration of aniracetam normalize receptor function so that continued treatment is not necessary, or are the beneficial effects of aniracetam more pharmacological in nature so that the drug must be present in order to improve cognitive performance of injured rats? Thus, this experiment examined whether the compound must be biologically active in the organism during the time of testing in order to provide cognitive enhancement.

Procedure. Injured rats were treated with 50 mg/kg of aniracetam (n = 9) or vehicle (n = 8) beginning 24 hrs after injury and daily until day 15. MWM performance was assessed on days 16–20 (without aniracetam) after TBI. For comparison purposes, the MWM data from sham-injured rats in Experiment 1 were included.

Results. The mean latencies to reach the goal platform are presented in Figure 3. These data were analyzed by a 3 (group) × 5 (day) split-plot analysis of variance (ANOVA). The ANOVA indicated a significant main effect of group (F2,24 = 3.33, p < 0.05). Duncan’s Multiple Range test indicated that compared to sham-injured rats, injured-vehicle-treated rats demonstrated significantly impaired MWM performance (p < 0.05). The injured-vehicle-treated group did not differ from the injured group treated with 50 mg/kg of aniracetam that terminated prior to maze testing. Analysis of swim speed found no group differences (data not shown).

DISCUSSION

These results demonstrate the efficacy of using a positive modulator of AMPA receptor function as a delayed, chronic treatment for TBI-induced cognitive impairment. The results of Experiment 1 demonstrated that aniracetam (in doses of 25 or 50 mg/kg) is effective in reducing the cognitive deficits produced by TBI when treatment is initiated 24 h after TBI and continued throughout cognitive testing. Experiment 2 found that aniracetam is still effective in attenuating trauma-induced cognitive impairment even when the treatment is delayed for 11 days after injury. In fact, comparing the efficacy of aniracetam when it is administered soon after the injury (24 h in Experiment 1) or when it was delayed for 11 days after injury (Experiment 2), aniracetam is equally effective in reducing trauma-induced MWM deficits. This long therapeutic window for aniracetam is quite important. A number of post-traumatic pharmacological interventions that are beneficial to recovery have been started within 24 h of TBI. In fact, two experiments that have explicitly tested the effectiveness of delayed (>24 h) post-injury treatment have found that drugs that were effective when administered beginning 24 h after injury are no longer effective if delayed for 11 days after injury (O’Dell and Hamm, 1995; Pike and Hamm, 1995). Thus, the observation that aniracetam is an efficacious treatment when delayed for 11 days is an important finding.

In Experiment 3, when aniracetam was initiated 24 h after TBI, but drug treatment terminated prior to MWM testing, aniracetam-treated rats did not perform significantly better than injured-vehicle-treated rats. These results indicate that aniracetam improves cognitive function only when the drug is biologically available during behavioral testing in the water maze. Although few experiments have

FIG. 3. Mean goal latencies (±SEM) for each group on days 16–20 when aniracetam treatment was terminated prior to Morris Water Maze (MWM) testing. Injured animals treated with vehicle evidenced longer goal latencies than sham-injured animals (p < 0.05). Injured animals treated with 50 mg/kg of aniracetam chronically prior to, but not during, MWM testing did not significantly differ from injured animals treated with vehicle.
tested the effects of terminating pharmacological treatment, one previous study provides data relevant to the consequences of the termination of an efficacious drug intervention. Zhu et al. (2000) examined the effect of the dopamine enhancer l-deprenyl on cognitive function and neuroplasticity following TBI. The drug was administered beginning 24 h after injury and was terminated after 7 days. When rats were tested in the MWM 4 days after drug termination, significant cognitive improvement relative to untreated injured animals was observed following l-deprenyl treatment. l-Deprenyl treatment also attenuated the injury-induced loss in dopamine beta-hydroxylase immunoreactivity and acetylcholinesterase histochemistry staining. These results suggest that l-deprenyl-induced dopaminergic/noradrenergic enhancement facilitated cognitive recovery after brain injury, and it appears that l-deprenyl produced its beneficial cognitive effects via enhanced post-injury plasticity rather than the direct receptor simulation at the time of MWM testing.

Experiment 3 evaluated the possibility that the therapeutic effect of aniracetam was the result of a drug-enhanced remodeling of the neural network after injury, similar to that observed by Zhu et al. (2000). There is some evidence that aniracetam has a neuroplastic effect. For example, Fushiki et al. (1995) found that aniracetam stimulated neurite extension in cultured granule neurons. Thus, aniracetam’s beneficial effects on cognitive recovery may be mediated by a drug-induced neuroplasticity effect. The results of Experiment 3 argue against such an effect of aniracetam. In Experiment 3, contrary to the results reported with l-deprenyl, the termination of chronic treatment prior to cognitive testing resulted in aniracetam being ineffective in improving the cognitive performance of injured animals. This result suggests that aniracetam’s efficacy is the consequence of its pharmacologically mediated effects at the time of cognitive testing, via the drug’s CNS stimulatory effects.

Aniracetam’s beneficial effects on neuronal function could be mediated via multiple mechanisms of action. In addition to aniracetam’s well-documented effect on AMPA receptor desensitization, aniracetam has been shown to enhance long-term potentiation (LTP) (Satoh et al., 1986). Another effect of aniracetam is through its enhancement of glucose availability and ACh synthesis in the brain. Ouchi et al. (1999) found that the administration of aniracetam following lesion to the basal forebrain prevents the reduction in glucose metabolism typically observed after the lesion. TBI is also known to result in a period of depressed glucose metabolism following injury (Yoshino et al., 1991). Thus, aniracetam may improve behavioral function by increasing post-injury glucose metabolism.

Aniracetam has also been found to positively modulate several neurotransmitter systems, and as reviewed previously, research indicates that a number of receptor systems are hypofunctional chronically after TBI. There is increasing evidence that aniracetam has a positive interaction with central glutamatergic systems, potentiating endogenous glutamate release (Togashi et al., 2002). Aniracetam also enhances ACh release (Nakamura and Shirane, 1999) by means of aniracetam-elicited glutamate stimulating group II metabotropic glutamate receptors (Togashi et al., 1996). In addition, administration of aniracetam to stroke-prone spontaneously hypertensive rats (SHRSP) enhanced both DA and 5-HT release, ameliorating dopaminergic hypofunction observed in SHRSP rats. These results suggest that a glutamatergic mechanism may underlie aniracetam’s effect on other neurotransmitters, including ACh, DA, and 5-HT. Thus, in addition to aniracetam’s effect as an inhibitor of AMPA receptor desensitization, it stimulates the release of a number of other neurotransmitters, which may increase neuronal functioning and contribute to improved cognitive recovery.

If the behavioral deficits observed chronically after TBI are the result of the depression of normal neuronal activity, as proposed by the theory of diaschisis/RFD (Feeney, 1991), efficacious post-injury interventions would enhance neuronal activity. The results reviewed above indicate that aniracetam positively modulates excitatory neurotransmission through multiple mechanisms. Furthermore, these results indicate that diaschisis applies to neurochemical dysfunction as well as purely histological damage. Much of the previous research in support of diaschisis has been based on models with overt structural damage to the hippocampus or hippocampal pathways. However, neuronal cell death in the hippocampus is not a prerequisite for impaired cognitive function, and prolonged spatial memory deficits can occur in the absence of overt structural damage (Lyeth et al., 1990). Treatments that improve cognitive recovery under these circumstances are likely working through the activation of chronically depressed neuronal systems. This activation of neuronal function may underlie the beneficial effects that aniracetam has on cognitive recovery after TBI.

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REFERENCES


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