Nefiracetam improves Morris water maze performance following traumatic brain injury in rats

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Abstract

Nefiracetam, a pyrrolidone derivative, is a nootropic agent that has facilitated cognitive function in a wide variety of animal models of cognitive dysfunction. The purpose of this study was to investigate the efficacy of the chronic postinjury administration of nefiracetam (DM-9384) in improving cognitive performance following central fluid percussion brain injury in rats. Twenty-four hours following surgical preparation, a sham injury or a moderate fluid percussive injury (2.1 atm) was delivered. Nefiracetam was administered chronically (0 or 9 mg/kg, po, for sham animals and 0, 3, or 9 mg/kg for injured animals) on postinjury days 1–15. Cognitive performance was assessed using the Morris water maze (MWM) on postinjury days 11–15. Chronic administration of 3 and 9 mg/kg nefiracetam attenuated MWM deficits produced by central fluid percussive brain injury. Importantly, the MWM performance of the injured animals treated with 9 mg/kg did not significantly differ from uninjured, sham animals. The 9-mg/kg dose of nefiracetam did not have a positive or negative effect on MWM performance of uninjured animals. The results of the present experiment suggest that a nootropic such as nefiracetam may be an appropriate treatment for trauma-induced cognitive dysfunction. © 2001 Elsevier Science Inc. All rights reserved.

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1. Introduction

One of the most serious and persistent sequelae associated with both clinical (Brooks et al., 1987; Capruso and Levin, 1992) and experimental (Hamm et al., 1992, 1993; Lyeth et al., 1990) traumatic brain injury (TBI) is the impairment of cognitive function. Although the molecular mechanisms that underlie the chronic deficits in cognitive performance are poorly understood, the limited information available supports the hypothesis that following TBI, the neurotransmitter systems critical to learning and memory become hypofunctional. For example, several experiments have found a reduction in choline acetyltransferase (ChAT) following TBI (Leonard et al., 1994). Additionally, both high-affinity choline uptake (Dixon et al., 1995a,b) and scopolamine-evoked release of ACh are reduced after trauma (Dixon et al., 1995b). Additional support for the chronic suppression of neuronal function following TBI is found in studies of the effectiveness of postinjury pharmacological interventions targeted at specific receptor systems. For instance, pharmacological interventions that increased the availability of the precursor to ACh (Dixon et al., 1995b) or stimulated cholinergic receptors (Pike and Hamm, 1997a) have improved cognitive performance after TBI. Similarly, pharmacological treatments that positively modulate the glutamatergic (Temple and Hamm, 1996) or dopaminergic receptor systems (Dixon et al., 1999; Kline et al., 2000; Zhu et al., 2000) have also attenuated the cognitive deficits produced by TBI. This research indicates that increasing the posttraumatic activity of selected receptor systems is an effective treatment for improving cognitive performance after injury. However, because optimal cognitive function is dependent on the activity of numerous receptor systems, a posttraumatic pharmacological inter-
vention that enhances the activity of multiple receptor systems may be more efficacious in improving cognitive function than interventions targeted at a specific neurotransmitter system.

Nefiracetam, a pyrrolidine derivative, is a nootropic agent that has facilitated cognitive function in a wide variety of animal models of cognitive dysfunction. For example, nefiracetam reduced the amnesia produced by scopolamine (Sakurai et al., 1989) and apomorphine (Nabeshima et al., 1994). Nefiracetam has also been found to attenuate the amnesia produced by the neurotoxin AF64A (Abe et al., 1994), electrolytic lesion of the basal forebrain (Nishizaki et al., 1998), and hypoxia (Hiramatsu et al., 1992). Lastly, nefiracetam has improved the cognitive performance of aged rats (Nabeshima, 1994). Because nefiracetam is effective in several animal models of cognitive disorders, its mechanisms of action have been examined. Several studies have documented that pyrrolidine derivatives can activate multiple neurotransmitter systems, including dopaminergic (Funk and Schmidt, 1984), cholinergic (Spignoli and Pepeu, 1987), and glutamatergic receptor systems (Marchi et al., 1990). The improvement in cognitive function produced by nefiracetam has been attributed to increased release of neurotransmitters from presynaptic terminals (Funk and Schmidt, 1984; Marchi et al., 1990) or the enhancement of neurotransmitter release from postsynaptic sites (Isaakson and Nicoll, 1991; Ito et al., 1990; Tang et al., 1991). One possible mechanism by which nefiracetam facilitates the release of neurotransmitters is by the activation of the long lasting N/L-type Ca$^{2+}$ channel (Yoshi and Watabe, 1994).

If the cognitive deficits produced by TBI are the result of a suppressed or hypo-functional nervous system, then a pharmacological treatment such as nefiracetam that activates multiple neurotransmitter systems should attenuate the impairment. The purpose of the present experiment was to test the effectiveness of chronic, posttraumatic nefiracetam treatment on the recovery of cognitive function following experimental TBI in rats.

2. Methods

2.1. Subjects

Subjects were 39 adult male Sprague–Dawley rats (300–350 g) from Harlan Laboratories. Animals were housed individually in a standard vivarium with food and water ad libitum and a regulated 12/12 light/dark cycle. Subjects were randomly assigned to five groups: untreated sham-injured, sham-9 mg/kg, injured-saline, injured-3 mg/kg, injured-9 mg/kg. All procedures followed the guidelines established in the Guide for the Care and Use of Laboratory Animals (US Department of Health and Human Services) and were approved by our Institutional Animal Care and Use Committee.

2.2. Drug

Daichi Pharmaceutical graciously donated nefiracetam (DM-9384) for the purposes of this study. The drug was administered orally (doses: saline control, 3.0 mg/kg, 9.0 mg/kg) postinjury days 1 through 15 between 9:00 and 11:00 a.m. every morning. The injection volume was 1.0 ml/kg for all conditions. Previous studies using oral administration of nefiracetam allowed 15–60 min between oral injection and task assessment (Ito et al., 1990; Kline et al., 2000; Nabeshima et al., 1994; Oyaizu and Narahashi, 1999) as peak effects of nefiracetam occur within 2 h of administration (Pike and Hamm, 1997a). For this study, subjects were assessed on the cognitive task beginning 30 min postinjection.

2.3. Injury device and conditions

The fluid percussion device used to induce experimental brain injury (described in greater detail in Dixon et al., 1987) consisted of a Plexiglas cylindrical reservoir 60 cm in length and 4.5 cm in diameter. One end of the cylinder contained a rubber covered Plexiglas piston mounted in O-rings. A 2-cm long metal housing was mounted with an extracranial pressure transducer (Entran Devices, model EPN-0300*-100A) on the opposing end. A 5-mm tube (2.6 mm inner diameter) terminated with a male Leur-Loc fitting was secured at the end of the metal housing. This male Leur-Loc fitting connected to a female Leur-Loc fitting chronically implanted over the exposed dura mater of the rat (see Surgical Preparation). Double-distilled H$_2$O filled the entire system. To deliver the injury, a metal pendulum (4.54 kg), released from a controlled distance, impacted the piston of the injury device. The impact injected a small volume of H$_2$O into the closed cranial vault and produced a brief (~20 ms) displacement and deformation of neuronal tissue. The resulting pressure pulse was measured in atmospheres (atm) by the extracranial pressure transducer and was recorded on a storage oscilloscope (Tektronix 5111, Beaverton, OR).

2.4. Surgical preparation and injury

All rats were surgically prepared 24 h prior to injury. Following administration of anesthesia, sodium pentobarbital (54 mg/kg), animals were placed in a stereotaxic frame and the scalp sagittally incised. A 4.8-mm diameter central craniectomy (midway between bregma and lambda, centered over the central suture) was performed. Two nickel-plated screws (two 56 x 6 mm) were placed in burr holes 1 mm rostral to bregma and 1 mm rostral to lambda/1 mm medial to the lateral ridge. A modified Lucer-Loc syringe hub with a 2.6-mm inside diameter was placed over the exposed intact dura mater and bonded with cyanoacrylate adhesive and dental acrylic. Bacitracin was applied to the incision, the animal was carefully monitored until fully recovered, then returned to the vivarium with food and water ad libitum.
Twenty-four hours following surgical preparation, animals were anesthetized using 4% isoflurane in a carrier gas mixture of 70% N₂O and 30% O₂, and the scalp was incised to expose the hub. Injured group animals were connected to the injury device and the injury was delivered at 2.0 ± 0.1 atm. Sham injury groups followed precisely the same procedure as injured groups except the pendulum was not swung, thus no injury delivered. Bacitracin was applied and the wound was sutured closed. The animal was allowed to recover prior to being returned to the vivarium with food and water ad libitum.

Fluid percussion injury produces neurological signs of areflexia, unconsciousness, and stupor similar to those observed in humans (Brooks et al., 1987; Capruso and Levin, 1992). This type of injury also produces a range of cognitive deficits, including Morris water maze (MWM) performance, following injury (Gorman et al., 1993).

2.5. Behavioral measures

The posttraumatic suppression of the righting reflex was used to evaluate the injury severity between groups to ensure that animals in different treatment groups received an equivalent level of fluid percussion TBI. The MWM was used to assess cognitive performance following TBI.

2.5.1. Righting response

The righting response is a complex postural somatomotor function that is suppressed for several minutes following TBI. It was used as a measure of posttraumatic unconsciousness. Suppression of the righting response was evaluated by placing an animal on its back and recording the latency for the animal to right itself after injury.

2.5.2. Morris water maze

Assessment of cognitive function was measured using the MWM (Hamm et al., 1993). The apparatus consisted of a pool 180 cm in diameter and 60 cm in height located in a 2.5 × 2.5-m² room with numerous and permanent extra-maze cues (e.g., pipes, bookshelves). A clear Plexiglas platform was placed in one of four imaginary pool quadrants. The pool was filled with water to a level of 2.5 cm above the top of the platform to render it invisible to the animal. Water temperature was kept at approximately 22 ± 2°C. Animals were tested on days 11-15 after injury to allow for recovery of residual motor deficits. Rats were given four trials per day for five consecutive days. At each trial, the rats were placed by hand in the pool at one of four start locations (north, south, east, west) facing the wall. Start locations were randomly assigned to each animal. A computerized video tracking system (PolyTrack 4.01, San Diego Instruments, San Diego, CA) was used to record the animal’s latency to reach the goal. The tracking program also calculated the distance from the animal to the goal during each trial (at 0.2-s intervals) and added these distances together as a measure of how close the animal was swimming to the goal during the trial. This measure was defined as “cumulative distance from the goal.” To assess the possible confounding effects of motor impairment (either from TBI or drug treatment), swim speeds were also measured on each trial. Rats were given a maximum of 120 s to find the hidden platform. If an animal failed to find the platform after 120 s, it was placed on the platform by the experimenter. All rats were allowed to remain on the platform for 30 s and then were placed in a heated incubator between trials. There was a 4-min intertrial interval.

3. Results

3.1. Neurological assessment

A one-way ANOVA was performed to determine differences in duration of the suppression of the righting reflex among all injured groups. Fig. 1 depicts the significant main effect of Group found for suppression of the righting reflex [F(4,34) = 6.885, P < .001]. Injured animals showed significantly longer suppression of the righting reflex than sham-injured animals [F(1,42) = 36.107, P < .001]. Injured groups did not differ in duration of suppressed righting reflex [F(2,21) = 0.129, P > .05]. These results indicate injured groups received an equivalent level of injury severity.

3.2. Morris water maze assessment

3.2.1. Goal latency

Shorter goal latency indicates better performance in the MWM. Fig. 2 illustrates the mean latency to find
group contrasts indicated that there was no significant treatment effects. MWM Cumulative Distance 30000 -<> - Sham-sal ..••. Sham..Q.O ---+-lnj-5a1 -O--lnj-3.0 ---A----lnj-9.0 25000 ~ 20000 • 15000 j 10000 ~ 5000 0 11 12 13 15 Post.Injury Day Fig. 3. The effects TBI and nefiracetam on cumulative distance from the goal for all groups over the 5 days of MWM testing. Saline or nefiracetam (3 or 9 mg/kg) was administered beginning on day 1 after injury and continued for the duration of the experiment. Each point with a vertical bar on the graph represents the mean±S.E.M. for each group: Sham-Sal = sham-injured rats treated with saline; Sham-9.0 = sham-injured rats treated with 9 mg/kg nefiracetam; Inj-Sal = injured rats treated with saline; Inj-3.0 = injured rats treated with 3 mg/kg nefiracetam; Inj-9.0 = injured rats treated with 9 mg/kg nefiracetam.

the goal platform in the MWM procedure on days 11–15 after injury. A split-plot ANOVA (Group x Day) revealed a significant effect of group \(F(4,34)=11.10, P<.001\), day \(F(4,136)=52.48, P<.0001\), and a Day x Group interaction \(F(16,136)=1.89, P<.03\). Tukey's HSD tests were performed to determine group differences \((P<.05\) for each comparison). The results of these group contrasts indicated that there was no significant difference between the sham groups treated with saline or 9 mg/kg of nefiracetam. Injured animals treated with 3 mg/kg and injured animals treated with 9 mg/kg both had significantly shorter goal latencies than injured animals treated with saline. In addition, the MWM performance of animals treated chronically with 9.0 mg/kg nefiracetam following injury did not differ from uninjured control animals.

3.2.2. Cumulative distance

Since the cumulative distance calculates the distance the rat is from the goal during the trial, smaller numbers indicate better performance (Fig. 3). An ANOVA comparing injured to sham animals revealed a significant effect of injury on cumulative distance from the goal \(F(4,34)=8.69, P<.001\), day \(F(4,136)=32.85, P<.0001\), and a Day x Group interaction \(F(16,136)=2.65, P<.001\). Tukey's HSD tests were performed to determine group differences on cumulative distance from the goal \((P<.05\) for each comparison). The results of these group contrasts indicated that there was no significant difference between the sham groups treated with saline or 9 mg/kg of nefiracetam. Injured animals treated with 3 mg/kg and injured animals treated with 9 mg/kg both had significantly shorter cumulative distances from the goal than injured animals treated with saline.

3.2.3. Swim speed

A single-factor ANOVA (group) revealed none of the groups' swim speeds significantly differed \(F(4,34)=1.847, P<.186\). These results indicate differences in goal latency were not due to injury-induced motor impairment or drug treatment effects.

Fig. 3. The effects TBI and nefiracetam on cumulative distance from the goal for all groups over the 5 days of MWM testing. Saline or nefiracetam (3 or 9 mg/kg) was administered beginning on day 1 after injury and continued for the duration of the experiment. Each point with a vertical bar on the graph represents the mean±S.E.M. for each group: Sham-Sal = sham-injured rats treated with saline; Sham-9.0 = sham-injured rats treated with 9 mg/kg nefiracetam; Inj-Sal = injured rats treated with saline; Inj-3.0 = injured rats treated with 3 mg/kg nefiracetam; Inj-9.0 = injured rats treated with 9 mg/kg nefiracetam.
4. Discussion

This investigation tested the effectiveness of nefiracetam, a nootropic with diverse actions, on MWM performance after central fluid percussion TBI in rats. Results of the present experiment demonstrated that daily (days 1–15) postinjury oral administration of 3 or 9 mg/kg of nefiracetam was effective in improving MWM performance of brain-injured rats, and the goal latency of injured animals treated with 9 mg/kg of nefiracetam did not differ for sham-injured control animals. In addition, the 9-mg/kg dose of nefiracetam did not have any positive or negative effects on the MWM performance of uninjured, sham animals. Previous research reports have documented the effects of nefiracetam after various types of brain injury. For example, nefiracetam has improved the retention of a passive avoidance task following electroconvulsive shock (Hiramatsu et al., 1992, 1997).

Similarly, the amnesia produced by ischemia has also been ameliorated by nefiracetam (Nabeshina, 1994). The mechanisms by which nefiracetam improves cognitive performance after brain injury are uncertain. However, it is known that piracetam-like nootropics increase the activity of several neurotransmitter systems that are essential for cognitive function, including dopaminergic (Funk and Schmidt, 1984), cholinergic (Spignoli and Pepeu, 1987), and glutamatergic receptor systems (Marchi et al., 1990). Additionally, nefiracetam may facilitate the release of neurotransmitters through the activation of N/L-type Ca2+ channels (Yoshi and Watabe, 1994). Previous research has demonstrated that the selective manipulation of these same neurotransmitter systems that are affected by nefiracetam can improve cognitive performance after TBI. For example, the positive modulation of dopaminergic function with methylphenidate (Kline et al., 2000), amantadine (Dixon et al., 1999), or t-deprenyl (Zhu et al., 2000) has been found to reduce the deficits in MWM performance observed after TBI. Similarly, several experiments have found that enhancing the cholinergic receptor system by the administration of additional precursors also improved MWM performance of injured animals (Dixon et al., 1995b).

Pharmacological interventions that have augmented cholinergic neurotransmission by either blocking the muscarinic cholinergic autoreceptor (Pike and Hamm, 1997a) or activating the postsynaptic muscarinic receptor (Pike and Hamm, 1997b) have attenuated the deficits in MWM performance produced by TBI. The positive modulation of the glutamatergic system with n-cycloserine has also proven to be an effective treatment, attenuating MWM dysfunction after TBI (Temple and Hamm, 1996). It appears nefiracetam may share many of the same neurotransmitter effects that have been demonstrated to improve cognitive performance after TBI.

While nefiracetam shares many of the neurotransmitter effects that have been found to improve cognitive performance after TBI, nefiracetam has also been shown to affect a novel neurotransmitter system that has not been previously linked to cognitive deficits after TBI. Several experiments documented that nefiracetam stimulates nicotinic ACh receptors (Nishizaki et al., 1998; Nomura and Nishizaki, 2000; Oyaizu and Narahashi, 1999). Although the modulation of nicotinic receptors has been shown to be successful in enhancing cognitive function in a number of different experimental procedures, the specific role of nicotinic ACh receptors in cognitive impairment following TBI has not been examined and may be a productive new area of inquiry.

At this initial stage of research in posttraumatic pharmacological interventions, it is not clear what mechanisms are responsible for the improved cognitive outcome. However, as early as 1905, von Monakow (1969) hypothesized that the CNS enters a state of “functional depression” following insult. This theory of diaschisis has recently 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. Thus, according to theories of diaschisis and RFD, behavioral recovery should be associated with the dissipation of this neuronal depression. In other words, if neuronal function is depressed chronically after TBI, then interventions that are initiated during the chronic phase should be designed to increase neuronal activity in order to return neuronal function to the normal range. The results of the present experiment support the theories of diaschisis and RFD in that nefiracetam, which is known to enhance the activity of several neurotransmitter systems, is effective in improving behavioral function after injury. Because nefiracetam is effective in improving performance in numerous models of cognitive dysfunction, Sarter (1991) questioned whether nefiracetam was a “universal attenuator.” Because the cognitive impairment that is produced by TBI is not the result of the dysfunction of one neurotransmitter system but is more likely the consequence of the impairment of multiple neurotransmitter systems, a pharmacological intervention that increases the activity of several neurotransmitters should be effective. Thus, as suggested by our results, a “universal stimulator” of neurotransmission such as nefiracetam may be an appropriate treatment for trauma-induced cognitive dysfunction.

References


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