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Effect of Tetrahydroaminoacridine, a Cholinesterase Inhibitor, on Cognitive Performance Following Experimental Brain Injury

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ABSTRACT

An emerging literature exists in support of deficits in cholinergic neurotransmission days to weeks following experimental traumatic brain injury (TBI). In addition, novel cholinomimetic therapeutics have been demonstrated to improve cognitive outcome following TBI in rats. We examined the effects of repeated postinjury administration of a cholinesterase inhibitor, tetrahydroaminoacridine (THA), on cognitive performance following experimental TBI. Rats were either injured at a moderate level of central fluid percussion TBI (2.1 ± 0.1 atm) or were surgically prepared but not delivered a fluid pulse (sham injury). Beginning 24 h after TBI or sham injury, rats were injected (IP) daily for 15 days with an equal volume (1.0 ml/kg) of either 0.0, 1.0, 3.0, or 9.0 mg/kg THA (TBI: n = 8, 8, 10, and 7, respectively, and Sham: n = 5, 7, 8, 7, respectively). Cognitive performance was assessed on Days 11–15 after injury in a Morris water maze (MWM). Analysis of maze latencies over days indicated that chronic administration of THA produced a dose-related impairment in MWM performance in both the injured and sham groups, with the 9.0 mg/kg dose producing the largest deficit. The 1.0 and 3.0 mg/kg doses of THA impaired MWM performance without affecting swimming speeds. Thus, the results of this investigation do not support the use of THA as a cholinomimetic therapeutic for the treatment of cognitive deficits following TBI.

Key words: cholinergic; cholinesterase; Morris water maze; muscarinic; rats; tacrine (THA); traumatic brain injury; treatment

INTRODUCTION

One of the most debilitating consequences of traumatic brain injury (TBI) is an enduring impairment of cognitive function. For example, mnemonic deficits are a common long-term sequelae of clinical closed head injuries (Brooks et al., 1987; Capruso and Levin, 1992; Levin, 1992; Oddy et al., 1985; Schmitter–Edgecombe et al., 1992) and are also a prominent feature in animal models of TBI (Hamm et al., 1993; Hamm et al., 1992; Lyeth et al., 1990; Pierce et al., 1994; Smith et al., 1991). Despite the frequency and persistence of cognitive dysfunctions following TBI, few experiments have investigated the pharmacotherapeutic attenuation of

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cognitive deficits during the recovery period following experimental head injury (Liu et al., 1993; O'Dell and Hamm, 1995; Pierce et al., 1993; Pike and Hamm, 1995; 1997; Temple and Hamm, 1996). The analysis of neuropharmacological and neurochemical correlates to cognitive deficits during recovery from TBI can provide potential strategies for the treatment of cognitive impairment associated with TBI.

A cholinergic insufficiency hypothesis has been advanced to explain, at least in part, the long-term deficits in cognitive function that have been observed following experimental TBI (Dixon et al., 1995a, 1995c; Hamm et al., 1994; O'Dell and Hamm, 1995; Pike and Hamm, 1995). Support for this hypothesis is demonstrated by a posttraumatic decrease in basal forebrain choline acetyltransferase (ChAT) immunoreactivity (IR) (Dixon et al., 1995b; Leonard et al., 1994; Schmidt and Grady, 1995; Sinson et al., 1995) as well as decreased hippocampal cholinesterase (AChE) terminal density (Grady et al., 1992) at time points that are correlated with impaired cognitive performance in rodent TBI (Hamm et al., 1993; Hamm et al., 1992; Lyeth et al., 1990). In addition, anticholinergic scopolamine potentiates cognitive impairment following TBI in rats (Dixon et al., 1994) and reduces evoked release of acetylcholine (ACh) *in vivo* at 2 weeks after experimental TBI (Dixon et al., 1995a). Thus, if a trauma-induced cholinergic insufficiency contributes to cognitive impairment after brain injury, then increasing cholinergic tone may have beneficial results on outcome. Recently, administration of pharmacologic agents designed to enhance cholinergic neurotransmission have been shown to attenuate cognitive deficits in rodent models of TBI (Liu et al., 1993; O'Dell and Hamm, 1995; Pike and Hamm, 1995; 1997).

Tetrahydroaminoacridine (THA, Tacrine™) is a reversible AChE inhibitor that has recently been approved for clinical treatment in mild to moderate Alzheimer's disease (AD) patients (Enz et al., 1993; Knapp et al., 1994). Cholinesterase inhibitors such as THA inhibit the enzymatic catabolism of ACh, thus maintaining synaptic residence of ACh. THA administration has been reported to have beneficial effects on cognitive outcome in animals model of AD (Hodges et al., 1990; Kwo-On-Yeun et al., 1990; Ueki and Miyoshi, 1989) and in patients diagnosed with AD (Davis et al., 1992; Eagger et al., 1992; Farlow et al., 1992; Gaithier et al., 1990; Molloy et al., 1991). Because an enduring impairment in cognitive function is a prominent sequelae of human and experimental TBI, and cholinergic neurochemical deficits may contribute to TBI-induced cognitive impairment, we tested the hypothesis that the AChE inhibitor, THA, would improve cognitive performance following central fluid percussion TBI in rats.

MATERIALS AND METHODS

Subjects

Sixty-eight male Sprague-Dawley rats (Hilltop Lab Animals, Inc., Scottsdale, PA) weighing 300–350 g were used in the study. Rats were individually housed (at 20°–22°C and 06:00 to 18:00 L:D cycle) with free access to food and water.

Drug/Vehicle Preparation

Tetrahydroaminoacridine (Cat. No. A-100) was purchased from Research Biochemicals Incorporated (Natick, MA) and was dissolved in an isotonic saline solution. Animals injected with vehicle were given an equal volume of the vehicle. Injection volume was 1 ml/kg for all conditions.

Injury Device and Conditions

The fluid percussion device used to produce experimental brain injury was identical to that used previously on rodents and described elsewhere in greater detail (Dixon et al., 1987). Briefly, the device consisted of a Plexiglas cylindrical reservoir 60 cm long and 4.5 cm in diameter. One end of the cylinder contained a rubber covered Plexiglas piston mounted in O-rings. The opposite end of the cylinder had a 2-cm long metal housing mounted with an extracranial pressure transducer (Entran Devices, Inc., model EPN-0300*-100A). Fitted at the end of the metal housing was a 5-mm tube with a 2.6-mm inner diameter that terminates with a male Leur-Loc fitting. This fitting was connected to a female Leur-Loc fitting that was chronically implanted over the exposed dura mater of the rat. The entire system was filled with isotonic saline. The injury was produced by a metal pendulum that strikes the piston of the injury device. The resulting impact injected a small volume of saline into the closed cranial vault and produced a brief (≈20 msec) displacement and deformation of neuronal tissue. The resulting pressure pulse was measured in atmospheres (atm) by the extracranial transducer and recorded on a storage oscilloscope (Tektronix TDS-340, Beaverton, OR).

Surgical Preparation and Injury

All animals were surgically prepared under sodium pentobarbital anesthesia (54 mg/kg) 24 h before fluid percussion injury. Animals were placed in a stereotaxic frame and the scalp was sagittally incised. A 4.8 mm diameter central craniectomy was performed over the sagittal suture midway between bregma and lambda. Two nickel-plated skull screws (2–56 × 6 mm) were placed

in burr holes 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, intact dura mater, and bonded in place with cyanoacrylate adhesive and dental acrylic. After the acrylic hardened, the injury tube was plugged with Gelfoam and the scalp sutured closed over the injury tube. Bacitracin was applied to the incision, and the animal was returned to its home cage.

Twenty-four hours after surgical preparation animals were anesthetized with 4% isoflurane in a carrier gas of 70% N_2O and 30% O_2 and injured at a moderate level of central fluid percussion injury (2.1 ± 0.1 atm as recorded by the transducer). With the central fluid percussion model, this injury magnitude is not associated with overt neuronal cell death, axonal injury, or ischemia (Chou et al., 1991; DeWitt et al., 1988; Lyeth et al., 1990) and produces acute hypotension, bradycardia, and increased plasma glucose levels (Dixon et al., 1987). Central fluid percussion injury produces neurological signs of areflexia, unconsciousness, and stupor similar to that observed in other species and humans (Dixon et al., 1987; Lyeth et al., 1988). In addition, motor deficits last 5–7 days after injury and cognitive impairment is present for weeks (Dixon et al., 1987; Hamm et al., 1992; Lyeth et al., 1990). The experimental procedures have been reviewed and approved by our institution's Animal Care and Use Committee.

Morris Water Maze Procedure

The Morris water maze (MWM) (Morris, 1981) was used to assess cognitive performance following TBI. The MWM procedure employed a 180-cm diameter and 60-cm high metal pool painted white and filled with water to a depth of 27 cm. Water temperature was maintained at 23°–26°C throughout the duration of water maze testing. A clear, Plexiglas platform 10 cm in diameter and 25 cm high (i.e., 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 extra-maze cues (e.g., windows, pipes, bookcase) that remained constant throughout the experiment.

The Morris water maze procedure consisted of 4 trials per day for 5 consecutive days (Days 11–15 after injury). On each trial, rats were placed in the pool by hand at 1 of 4 start locations. The starting locations were separated by 90° and were identified as south, west, north, and east. Rats started a trial once from each of the 4 possible start locations on each day. The order of starting locations was randomized for each animal on each day. The goal platform was positioned 45 cm from the outside wall in the southeast quadrant of the maze for all groups. The latency to find and mount the hidden platform was used as the primary dependent variable. Swimming speeds were also recorded to assess drug-induced motor effects. Rats were given a maximum of 120 s to find the hidden platform. If the rats failed to find the platform after 120 s, they were placed on the platform by the experimenter. All rats remained on the platform for 30 s before being placed in a heated incubator (30°C) between trials. There was a 4-min intertrial interval. Personnel evaluating animals in the MWM were blinded to the injury and drug treatments of each animal.

Experimental Design

This experiment was designed to test the effects of daily postinjury administration of THA on cognitive performance following TBI. Beginning 24 h after TBI or sham injury, all rats were injected (IP) daily with either saline, 1.0, 3.0, or 9.0 mg/kg THA (TBI: $n = 8, 8, 10,$ and 7, respectively, and Sham: $n = 5, 7, 8, 7$, respectively) for the duration of the experiment. On Days 11–15 postinjury, rats were injected 30 min prior to assessment in the MWM. The doses of THA employed in this study fall within a therapeutically effective range that have been previously shown to enhance memory in animals and humans (Hodges et al., 1990; Kiefer-Day and El-Fakahany, 1992; Nielson et al., 1989; Riekkinen et al., 1990; Summers et al., 1986). Daily administration of THA after injury was based on previous research on cognitive enhancement following TBI (Liu et al., 1993; O'Dell and Hamm, 1995; Pike and Hamm, 1997; Pike et al., 1995).

Statistical Analysis

A mean daily latency to find the goal platform during MWM testing on Days 11–15 postinjury was computed for each rat. A split-plot analysis of variance (ANOVA) Injury × Dose × Day ($2 \times 4 \times 5$) was used to analyze maze latencies. If a significant effect was found, separate split-plot ANOVAs were used for pairwise group contrasts. The Dunn–Sïdák multiple comparisons correction was used to control for multiple group contrasts. A significance level of $p < 0.05$ was used for all tests. The mean swim speed for each dose of THA was examined with a one-factor (dose) ANOVA.

RESULTS

MWM Performance–Goal Latency

Figure 1 illustrates the mean (+SEM) latency to find the goal platform on the MWM procedure on Days 11–15 for the TBI groups (A) and sham-injured groups (B). To

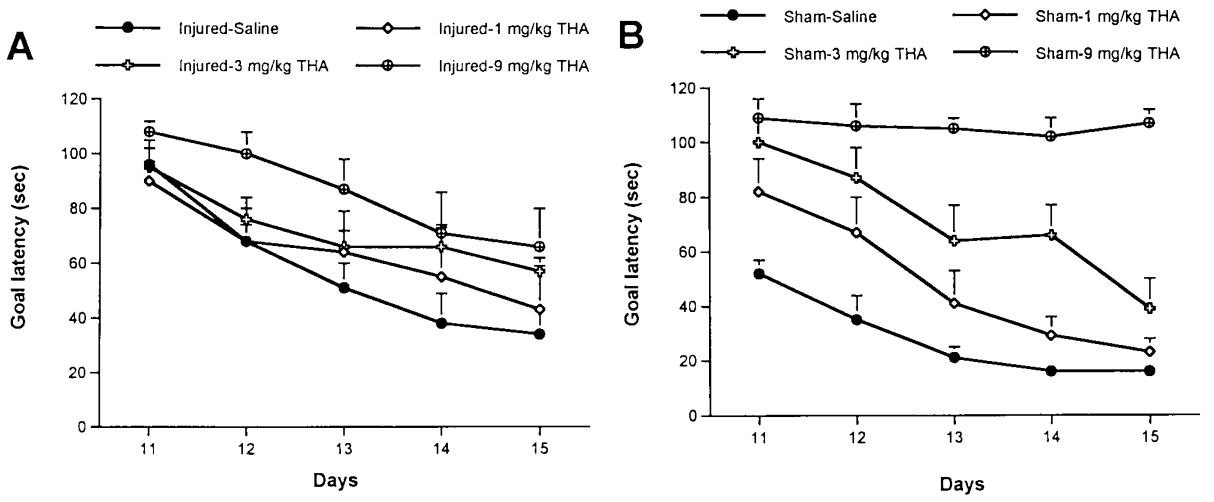

FIG. 1. Mean latencies (+SEM) to find the goal platform on Days 11–15 postinjury for the injured groups (A) and sham-injured groups (B). Daily postinjury administration of THA resulted in a dose-related impairment of maze performance for both injured and sham-injured animals ($p < 0.05$ for each comparison).

confirm that the fluid percussion injury produced the expected deficit in MWM performance, the injured saline-treated group was compared to the sham saline-treated group by a split-plot ANOVA (Group × Day). The main effect of Group was significant [$F(1,4) = 17.36$, $p < 0.002$], indicating that the fluid percussion injury did impair the performance of rats in the MWM. To examine the effects of THA treatment, a split-plot ANOVA (Injury × Dose × Day) revealed that the main effect for Injury was not significant. The main effect of Dose was significant [$F(3,59) = 18.32$, $p < 0.0001$] and indicated that there was a dose-dependent increase in goal latencies. Subsequent pairwise group contrasts indicated that all of the injured THA-treated groups performed equivalently or had significantly longer latencies than the injured saline-treated group. Injured animals treated with either the 1.0 mg/kg or the 3.0 mg/kg dose of THA were not significantly different from the injured saline-treated group [$F(1,14) = 0.068$, $p > 0.05$] and [$F(1,16) = 1.599$, $p > 0.05$], respectively). However, injured animals treated with 9.0 mg/kg of THA had significantly longer latencies in the MWM than the injured saline-treated group [$F(1,13) = 5.842$, $p < 0.05$]. In the sham-injured groups, each treatment group (i.e., saline, 1.0, 3.0, and 9.0 mg/kg) was significantly different from each other ($P < 0.05$ for each comparison). The Injury × Dose interaction was also significant [$F(3,59) = 3.83$, $p < 0.02$]. This interaction was the result of increasing doses of THA impairing maze performance more in the sham-injured groups than in the TBI groups. Pairwise group contrasts indicated that the 9 mg/kg dose of THA impaired the performance of sham-injured rats more than injured rats ($p < 0.05$). The main effect of Day was also significant [$F(4,236) = 43.87$, $p < 0.0001$], indicating that over days the goal latencies became shorter.

MWM Performance–Swim Speed

Fig. 2 illustrates the mean swim speed calculated for each dose of THA during MWM testing. The ANOVA on these data revealed a significant Dose effect [$F(3,51) = 21.36$, $p < 0.0001$]. Fot the sham and injured groups, post-hoc group comparisons indicated the 9.0 mg/kg dose of THA significantly reduced swim speed ($p < 0.01$) compared to saline, 1.0, and 3.0 mg/kg of

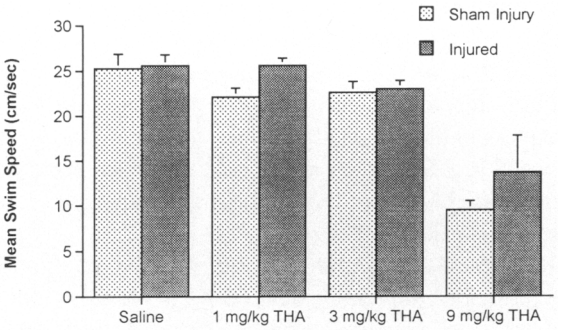

FIG. 2. Mean swimming speed (+SEM) of the injured and sham-injured groups for each dose of THA averaged over MWM testing on Days 11–15. For both the injured and sham-injured groups, the 9 mg/kg dose produced a significant decrease in swim speed relative to other doses ($p < 0.05$ for each comparison). The 1 and 3 mg/kg doses did not affect swim speed.

THA. The 1.0 and 3.0 mg/kg doses of THA did not affect swim speed when compared to saline for both groups.

DISCUSSION

Following moderate central fluid percussion TBI in rats, daily administration of the cholinesterase inhibitor THA (1.0, 3.0, or 9.0 mg/kg, Days 1–15 postinjury) did not improve MWM performance compared to injured-saline treated rats. In sham-injured rats, chronic treatment with THA produced a dose-dependent impairment in MWM performance. Moreover, the 9.0 mg/kg dose of THA was found to significantly decrease swimming speed. Other nootropic compounds also have altered the performance of sham-injured animals (Pierce et al., 1993; Temple and Hamm, 1996).

The inability of THA, at doses that do not affect swim speed, to ameliorate the deficit in MWM performance suggests that the chronic administration of THA does not appear to be an effective strategy for the attenuation of cognitive impairment following experimental TBI. Previous research has shown that a 0.3–3.0 mg/kg dose of THA can enhance memory in humans and animals (Hodges et al., 1990; Nielson et al., 1989; Summers et al., 1986) and a 3.0 mg/kg dose is typically the most effective dose found to attenuate water maze deficits following lesioning of the medial septum (Riekkinen et al., 1991; Riekkinen et al., 1990). Both a lower (1.0 mg/kg) and a higher (5.0 mg/kg) dose of THA have been shown to be ineffective in improving maze performance of medial septum lesioned rats (Riekkinen et al., 1991; Riekkinen et al., 1990). Furthermore, higher doses of AChE inhibitors have been shown to impair memory performance (Flood et al., 1981; Haroutunian et al., 1985; Santucci et al., 1989) or to be neurotoxic (Walker et al., 1995) and cause muscarinic receptor downregulation (Alonso et al., 1990; Flynn and Mash, 1989; Fortuna et al., 1994; Pintor et al., 1994).

The negative behavioral effects of THA observed in this study, particularly in the sham-injured rats, are consistent with reports that indicate repeated cholinesterase inhibition may reduce ACh synthesis via increased extracellular ACh concentrations competing for choline uptake sites (Becker et al., 1988; Brooks et al., 1987). Similarly, because presynaptic muscarinic M_2 autoreceptors regulate ACh release (Doods, 1995; Potter et al., 1984; Richards, 1990; Vizi et al., 1989), THA-induced increases in extracellular ACh levels may inhibit presynaptic ACh release. Because the sham-injured rats were more impaired by the 9 mg/kg dose of THA than the injured rats, the adverse effects of AChE inhibition were greater in uninjured rats. The differential effects of THA on injured and sham-injured rats may be the result of alterations in the cholinergic system produced by TBI. As was reviewed earlier, a number of experiments support a cholinergic insufficiency hypothesis after TBI (Dixon et al., 1994; 1995a; 1995b; Grady et al., 1992; Leonard et al., 1994; Schmidt and Grady, 1995; Sinson et al., 1995). If the injured animal's cholinergic system is dysfunctional, then THA's adverse effects may also be attenuated in injured animals compared to uninjured animals.

Because of the potential negative effects of AChE inhibitors like THA, it has been suggested that the use of selective cholinomimetic therapeutics, targeted at specific muscarinic receptor subtypes, may be a more beneficial strategy for enhancing cognitive performance in cases of cholinergic hypofunction (Doods et al., 1993a, 1993b; Doods, 1995; Moltzen and Bjornholm, 1995; Sarter et al., 1990).

For example, pharmacotherapeutic strategies for enhancing cholinergic neurotransmission include inhibition of the enzymes that catabolize ACh, increasing ACh release, or direct stimulation of M_1 type muscarinic receptors. However, clinical cholinomimetic therapies in AD have generally produced discouraging results (Gaithier et al., 1991; Gray et al., 1989; Whitehouse, 1988). Although the cholinesterase inhibitor THA has provided positive clinical results (Davis et al., 1992; Farlow et al., 1992; Summers et al., 1986) and is currently an approved pharmacological treatment for the dementia of AD, it too has shown equivocal effects in AD as well as in animal models of cholinergic hypofunction (Sarter et al., 1990). The relative failure of some types of cholinomimetic therapies may reflect poor receptor subtype specificity, unwanted peripheral side effects, and interactions with other neurotransmitter systems. For example, THA can act as an antagonist at the NMDA receptor (Albin et al., 1988) and it is known that an NMDA antagonist accelerates maze deficits in TBI rats (Hamm et al., 1994). Furthermore, treatment with cholinesterase inhibitors and nonselective muscarinic agonists results in prolonged tonic stimulation of postsynaptic receptors and inhibition of presynaptic release of ACh by stimulation of presynaptic M_2 receptors. Thus, AChE inhibitors and muscarinic agonists compromise the endogenous patterning of transmitter release that may interfere with optimal neuronal signalling. For instance, normal interneuronal communication is probably characterized by a complex and synchronous patterning of neurotransmitter signaling. Thus, the absolute concentrations of extracellular ACh are probably not as important to intercellular communication as is the maintenance of normal signal patterning (see Sarter et al., 1990 for a discussion of tonic stimulation vs. signal amplification).

Whereas THA was not effective in improving cogni-

tive performance after TBI, other more selective cholinomimetic interventions have been efficacious (O'Dell and Hamm, 1995; Pike and Hamm, 1995; 1997). For example, administration of compounds that are selective for presynaptic M_2 autoreceptors or antagonists/negative-modulators of the γ-aminobutyric acid$_A$ ($GABA_A$) receptor offer a promising alternative approach to amplification of meaningful cholinergic signalling. Selective M_2 antagonists and $GABA_A$ antagonist/negative-modulators may provide a more physiologically appropriate modulation of cholinergic transmission as this approach would theoretically amplify the endogenous release of ACh rather than providing continuous tonic stimulation of the postsynaptic receptors. Moreover, enhancement of presynaptic ACh release by blockade of M_2 autoreceptors also promotes stimulation of nicotinic receptors and the further release of ACh (Doods, 1995). Furthermore, the presynaptic localization of M_2 heteroreceptors on glutamatergic neurons results in the release of the excitatory amino acid glutamate after administration of M_2 antagonists (Marchi and Raiteri, 1989; Mrzljak et al., 1993; Vilaro et al., 1992). Excitatory amino acids have also been implicated in mediating cognitive processes and a deficit in glutamatergic neurotransmission has also been hypothesized to contribute to the cognitive deficits following TBI (Hamm et al., 1994; Temple and Hamm, 1996).

The recent development of muscarinic receptor subtype-selective compounds may prove beneficial in treating disorders that affect cognition including brain trauma and Alzheimer's disease. In addition, newer generations of cholinesterase inhibitors with fewer side effects may also prove to be more efficacious than THA. As suggested by Doods (1995), centrally acting and selective M_2 antagonists in conjunction with cholinesterase inhibitors may prove more efficacious for the treatment of cognitive disorders involving cholinergic deficits than current strategies that employ muscarinic agonists or cholinesterase inhibitors alone. It must also be noted that cognitive impairment following TBI is mediated by multiple neurotransmitter and other neurochemical alterations. Cholinergic enhancement is only one strategy by which recovery may be facilitated. Research on the complex mechanisms that maintain the long-term behavioral deficits after TBI will provide many possible pharmacotherapies that may have clinical relevance for the human head-injured patient.

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