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Activating the Posttraumatic Cholinergic System for the Treatment of Cognitive Impairment Following Traumatic Brain Injury

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PIKE, B. R. AND R. J. HAMM. *Activating the posttraumatic cholinergic system for the treatment of cognitive impairment following traumatic brain injury.* PHARMACOL BIOCHEM BEHAV **57**(4) 785–791, 1997.—Cognitive impairment after traumatic brain injury (TBI) is correlated with decreased cholinergic markers of neuronal viability. The purpose of this experiment was to test the hypothesis that pharmacological activation of the muscarinic cholinergic system during the recovery period after TBI will improve cognitive performance. LU 25-109-T is a partial muscarinic M_1 agonist that also acts as an antagonist at presynaptic M_2 autoreceptors (thus, increasing ACh release). Injured rats were injected subcutaneously daily for 15 days with either 0.0, 3.6, or 15 μmol/kg of LU 25-109-T beginning 24 h after a receiving a moderate (2.1 ± 0.1 atm) level of central fluid percussion brain injury. Cognitive performance was assessed on days 11–15 postinjury in a Morris water maze (MWM). Injured rats treated with 15 μmol/kg, but not those treated with 3.6 μmol/kg, showed a significant improvement ($p < 0.01$) in MWM performance as compared with injured vehicle-treated rats. This result supports the hypotheses that a decrease in posttraumatic cholinergic neurotransmission contributes to TBI-induced cognitive deficits and that increasing cholinergic tone during the recovery period following TBI will improve cognitive performance. © 1997 Elsevier Science Inc.

Lu 25-109-T M_1 agonist M_2 antagonist Morris water maze Muscarinic Rats Spatial memory Treatment

NUMEROUS investigations have documented the therapeutic effects of excitatory neurotransmitter antagonists on the prevention or reduction of secondary injuries that occur following experimental traumatic brain injury (TBI) (13,15,19, 23,26,40). In contrast, relatively few experiments have focused on the promotion of functional recovery during the days to weeks following experimental TBI. One of the most enduring and devastating sequelae associated with both clinical (3,5, 31,37) and experimental (14,18,24,39) brain trauma is the impairment of cognitive function. It has recently been hypothesized that a hypoactive neuronal cholinergic system may mediate, at least to some extent, the observed long-term cognitive deficits that occur after TBI (8,10,16,32,34). For example, a decrease in the number of basal forebrain choline acetyltransferase (ChAT)-immunoreactive (IR) perikarya has been observed following central (36) and lateral (20,36,38) fluid percussion TBI, as well as in a cortical impact model of TBI (9). In addition, the muscarinic cholinergic receptor (mAChR) antagonist scopolamine potentiates cognitive impairment in rats following TBI (10), and the evoked release of hippocampal acetylcholine (ACh) in vivo is also reduced at 2 weeks after experimental TBI (8).

If brain trauma causes a reduction in the synthesis and/or release of synaptic ACh, then selective cholinomimetic pharmacotherapies may restore some of the lost cognitive function during the recovery period following TBI. The recent development of cholinergic compounds that readily cross the blood–brain barrier (BBB) and that are highly selective for subtypes of muscarinic cholinergic receptors (mAChRs) provides a clinically relevant approach to cognitive enhancement. For instance, it has been suggested that administration of partial mAChR agonists that are selective for the postsynaptic M_1 mAChR, and that also display presynaptic M_2 antagonist effects, may provide a functional approach for the treatment of cognitive dysfunction (29). The use of partial mAChR agonists, rather than full agonists, may be useful in that they may

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avoid tonic stimulation of the postsynaptic M_1 receptor, which can interfere with cell signalling (35) and cause receptor downregulation (2,25,33). In addition, M_2 receptors are located primarily presynaptically, and antagonism of these receptors promotes the endogenous release of ACh (12).

The novel compound Lu 25-109-T [5-(2-ethyl-2H-tetrazol-5-yl)-1,2,3,6-tetrahydro-1-methylpyridine, L-(+)-tartate; 343.4 g/mol] is a partial agonist of the M_1 mAChR that also displays M_2/M_3 antagonist effects (27,28). Lu 25-109-T readily crosses the BBB and reaches maximal neuronal bioavailabilty within 10 min of subcutaneous (SC) administration. Behaviorally, Lu 25-109-T has been shown to significantly improve Morris water maze (MWM) performance in both young and age-impaired rats (27,28). Thus, the purpose of this investigation was to test the effects of pharmacological activation of the muscarinic cholinergic system with the cholinomimetic Lu 25-109-T on cognitive performance of rats following a moderate level of central fluid percussion traumatic brain injury.

METHOD

Subjects

Forty male Sprague–Dawley rats (Hilltop Lab Animals, Inc., Scottsdale, PA, USA) weighing 300–350 g were used in the study. Rats were individually housed (at 20–22°C and a 12 L:12 D cycle, lights on 0600–1800 h) with free access to food and water.

Drug/Vehicle Preparation

Lu 25-109-T was a gift from Dr. Eddi Meier, A/S Lundbeck, Copenhagen-Valby, Denmark. The compound was dissolved in an isotonic saline solution. Animals injected with vehicle were given an equal volume of the saline solution 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 (11). 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 terminated with a male Luer-Loc fitting. This fitting was connected to a female Luer-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 striking the piston of the injury device. The resulting impact injected a small volume of saline into the closed cranial vault and produced a brief ($\sim$20 ms) 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 5111, Beaverton, OR, USA).

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 $\times$ 6 mm) were placed in burr holes 1 mm rostral to bregma and 1 mm caudal to lambda. A modified Luer-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 was 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 $\pm$ 0.1 atm as recorded by the transducer) or were connected to the injury device but were delivered a fluid pulse (sham-injured control groups). With the central fluid percussion model, a 2.0–2.2-atm injury is not associated with overt neuronal cell death, axonal injury, or ischemia (6,7,24), but produces acute hypotension, bradycardia, and increased plasma glucose levels (11). Central fluid percussion injury produces neurological signs of areflexia, unconsciousness, and stupor similar to those observed in other species and humans (11,22). In addition, motor deficits last 5–7 days after injury, and cognitive impairment is present for weeks (11,18,24). The experimental procedures have been reviewed and approved by our institution's Animal Care and Use Committee.

Behavioral Measures

Posttraumatic suppression of the righting reflex, decrease in body weight, and impairment of rotarod performance were used as measures of injury severity among groups and to ensure that animals in different treatment groups received an equivalent level of fluid percussion TBI. The MWM (30) was used as a measure of cognitive performance following TBI.

Righting response. The righting response is a complex postural somatomotor function that is suppressed for several minutes immediately following TBI; it was used as a measure of traumatic unconsciousness (11). Suppression of the righting response was measured as the duration for an animal to right itself three consecutive times after being placed on its back following the injury.

Body weight. To ensure an equivalent severity of injury among injured groups of animals, daily postinjury body weights were recorded. Loss of body weight following injury is a general index of neurologic outcome. Loss of body weight is most pronounced within the first few days following injury. Thus, the mean body weight of the first 5 days postinjury was used as the dependent variable.

Rotarod duration. Fine motor coordination was assessed with the rotarod device (17). Briefly, the rotarod device consisted of a metal frame with a motorized, rotating assembly of rods. Two clear Plexiglas disks (45 cm diameter) were positioned on each side of the frame. The walking apparatus consisted of 18 rungs (1-mm-diameter stainless steel rods) arranged in cylindrical fashion. The animals were required to walk on the rungs as the frame rotated. An automated control box (Bodine Electric Company) located adjacent to the device contained controls for speed, direction of rotation, and braking. Rotational speeds ranged from 0 to a maximum of 30 rpm. The procedure involved placing each animal on the device with the frame remaining stationary for 5 s. The speed was increased to 3 rpm at 5 s and then steadily increased by 3 rpm at 10-s intervals until the maximum of 30 rpm was

reached. Animals remained on the device for a maximum of 120 s (25 s at 30 rpm). Trials were also terminated if the animals fell off the frame or gripped the frame and spun around for one complete revolution without attempting to walk on the rungs. Rats were trained on the rotarod task before surgery and were tested 2 h before injury and for 5 days after injury. Animals were given two trials per day. The mean rotarod duration for the first 5 days postinjury was used as the dependent variable.

Morris water maze procedure. The MWM (30) 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 extramaze cues (e.g., windows, pipes, bookcase) that remained constant throughout the experiment.

The MWM procedure consisted of four trials per day for five consecutive days (days 11–15 after injury). In each trial, rats were placed in the pool by hand at one of four 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 four 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. A computerized video tracking sytem (Poly-Track version 4.01, San Diego Instruments, San Diego, CA, USA) was used to record each animal's latency to reach the goal platform, swim pattern, and total distance swum. The latency to find and mount the hidden platform was used as the dependent variable. 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.

Experimental Design

This experiment was designed to test the effects of daily postinjury administration of Lu 25-109-T on cognitive performance following TBI. Beginning 24 h after fluid percussion injury, rats were injected SC daily with either 0.0, 3.6, or 15 μmol/kg Lu 25-109-T (n = 8, 6, and 9, respectively) for the duration of the experiment. Sham-injured animals were injected with either 0.0 or 15 μmol/kg Lu 25-109-T (n = 8 for each sham-injured control group). On days 11–15 postinjury, rats were injected 10 min prior to assessment in the MWM. The doses of Lu 25-109-T employed in this study have been previously shown to improve MWM performance in both young and age-impaired rats (27,28). Daily administration of Lu 25-109-T after injury was based on previous research on cognitive enhancement following TBI (21,32,34).

Statistical Analysis

Measures of the righting reflex, body weight, and rotarod duration were each analyzed by a single-factor analysis of variance (ANOVA). The mean latency for suppression of the righting reflex and the mean of the first five postinjury days for body weight and rotarod duration were used for each analysis. Tukey post hoc tests ($\alpha = 0.05$) were used to analyze differences revealed by significant ANOVAs. A mean daily latency to find the goal platform during MWM testing on days 11–15 postinjury was computed for each group. A split-plot ANOVA [5 (group) × 5 (day)] 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. To ensure that the latency measure of maze performance was not confounded by injury or drug treatment, swim speeds were calculated for each animal and analyzed by a single-factor ANOVA. A significance level of $p < 0.05$ was used for all tests.

RESULTS

Behavioral Measures

Righting response. The mean suppression of the righting response is shown in Fig. 1A. A one-way ANOVA showed a significant effect of injury on the suppression of the righting response [$F(4, 35) = 42.807$, $p < 0.00001$]. The post hoc comparisons revealed that each of the injured groups had a significantly longer suppression of the righting response due to injury as compared with each of the sham-injured control groups ($p < 0.01$ for each comparison). The results also revealed that there was no significant difference between any of the injured groups on righting response after injury ($p > 0.05$ for each comparison). Furthermore, there was no significant difference between either of the sham-injured control groups. These results indicate that all of the injured groups received an equivalent severity of injury.

Body weight. The mean body weights for the first 5 days postinjury are shown in Fig. 1B. A one-way ANOVA on body weight showed a significant effect of injury on the loss of body weight [$F(4, 35) = 5.630$, $p < 0.01$]. The post hoc comparisons revealed that all of the injured groups lost a significant amount of weight due to injury, as compared with each of the sham-injured control groups ($p < 0.05$). However, there was no significant difference in body weight among any of the injured groups ($p > 0.05$ for each comparison). Furthermore, there was no significant difference between either of the sham-injured control groups. These results also indicate that all of the injured groups received an equivalent severity of injury.

Rotarod duration. With the rotarod task, a longer duration indicates better performance. The mean durations for the first 5 days postinjury are shown in Fig. 1C. A one-way ANOVA on rotarod duration showed a significant effect of injury on duration of the rotarod task [$F(4, 35) = 8.718$, $p < 0.0001$]. The post hoc comparisons revealed that all of the injured groups had a significantly reduced duration on the rotarod task due to injury as compared with each of the sham-injured control groups ($p < 0.01$ as compared with sham-injured/vehicle-treated rats and $p < 0.05$ as compared with sham-injured/Lu 25-109-T-treated rats). However, there was no significant difference in rotarod duration among any of the injured groups ($p > 0.05$). These results for rotarod duration indicate that all of the injured groups received an equivalent severity of injury as produced by the fluid percussion device.

Morris water maze performance. With the MWM task, a shorter goal latency indicates better performance. Figure 2A illustrates the mean latency to find the goal platform in the MWM procedure on days 11–15 after injury. A split-plot ANOVA [5 (group) × 5 (day)] revealed that the main effect for group was significant [$F(4, 35) = 15.796$, $p < 0.00001$]. Subsequent pairwise group contrasts indicated that the injured group treated daily with 15 μmol/kg of Lu 25-109-T performed

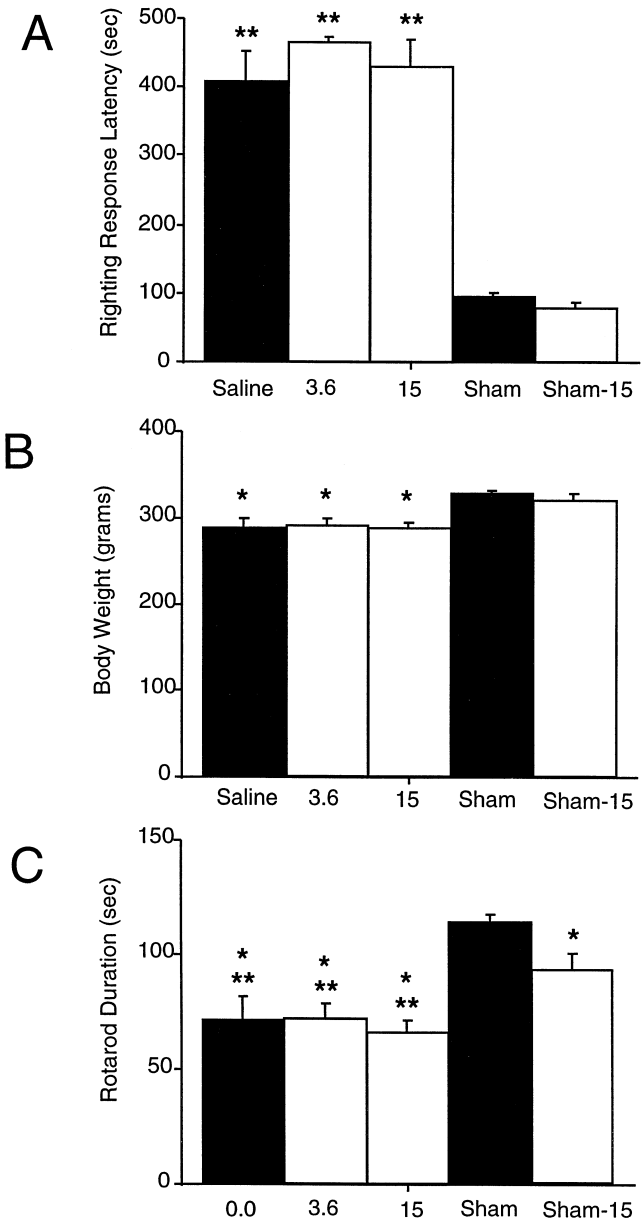

FIG. 1. Fluid percussion injury produced changes in righting response latencies, body weight, and rotarod durations of an equivalent magnitude among the injured groups treated with either 0.0, 3.6, or 15 μmol/kg Lu 25-109-T. This indicates that all of the injured groups received an equivalent severity of injury. (A) Each of the three injured groups differed significantly from sham-injured controls on suppression of the righting response (**$p < 0.01$ for each sham-injured group). (B) Following injury, each of the three injured groups lost a significant amount of body weight as compared with the sham-injured control groups (*$p < 0.05$). (C) Following injury, each of the three injured groups showed a significant impairment on the rotarod task as compared with the sham-injured control groups (**$p < 0.01$ as compared with sham-injured/vehicle-treated rats and *$p < 0.05$ as compared with sham-injured/Lu 25-109-T-treated rats).

significantly better in the MWM than did the injured group treated with vehicle (0.0 μmol/kg) [$F(1, 15) = 14.648, p < 0.01$]. Injured animals treated with 3.6 μmol/kg of Lu 25-109-T were found to perform no better in the MWM than did injured vehicle-treated rats [$F(1, 12) = 1.623, p = 0.2254$]. The results also indicated that the sham-injured rats treated with vehicle had significantly shorter goal latencies than did the injured rats treated with either the 0.0, 3.6, or 15 μmol/kg dose of Lu-25-109-T [$F(1, 14) = 67.861, p < 0.0001$; $F(1, 12) = 17.068, p < 0.01$; and $F(1, 15) = 13.028, p < 0.01$, respectively]. There was no significant difference in MWM performance between sham-injured rats treated with either vehicle or 15 μmol/kg Lu 25-109-T [$F(1, 15) = 0.208, p > 0.05$].

Figure 2B illustrates the mean swim speeds for each group. A single-factor ANOVA indicated that none of the groups' swim speeds were significantly different [$F(4, 27) = 0.321, p > 0.05$]. This result indicates that differences in maze latency were not due to injury-induced motor impairment or drug treatment effects.

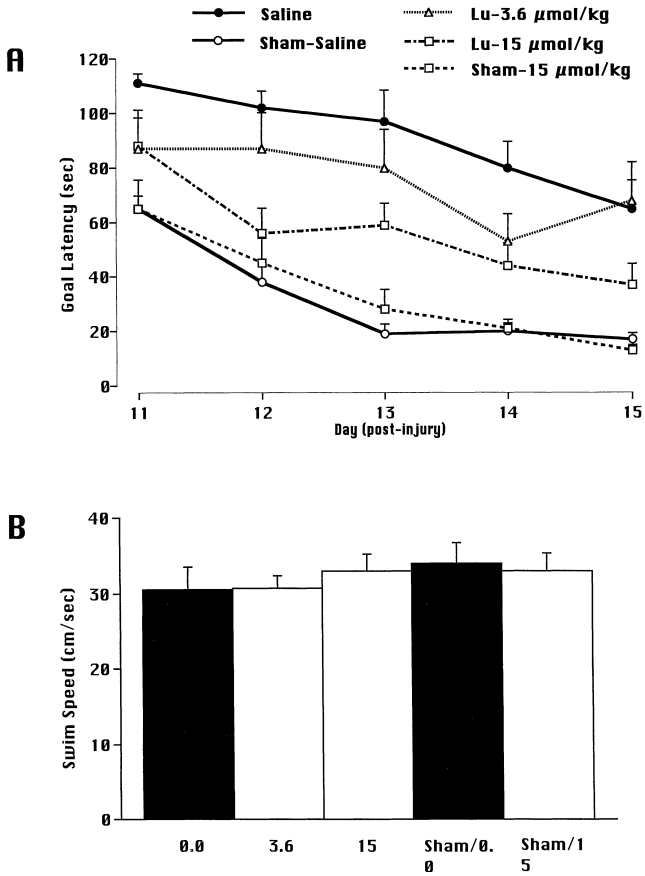

FIG. 2. (A) Mean (±SEM) latency to reach the goal platform in the MWM on days 11–15 following injury. Fluid percussion injury caused a significant impairment in MWM performance in each of the three injured groups as compared with the sham-injured control group ($p < 0.01$ for each comparison). However, injured animals treated with the 15 μmol/kg dose of Lu 25-109-T showed a significant improvement in MWM performance when compared with the injured vehicle-treated group ($p < 0.01$). The 15 μmol/kg dose of Lu 25-109-T had no effect on sham-injured animals when compared with the sham vehicle-treated animals ($p > 0.05$). (B) Swim speeds (±SEM) did not differ among groups, indicating that differences in maze latency were not due to injury-induced motor impairment or drug treatment effects.

DISCUSSION

This investigation tested the effects of Lu 25-109-T, a partial muscarinic M_1 receptor agonist that also displays muscarinic M_2/M_3 receptor antagonist properties, on MWM performance after central fluid percussion TBI in rats. Results of this study demonstrated that daily (15 days) postinjury SC administration of a 15-μmol/kg, but not a 3.6-μmol/kg, dose of Lu 25-109-T was effective in improving the cognitive performance of brain-injured rats on days 11–15 after experimental TBI. In addition, the 15-μmol/kg dose was found to have no effect on the MWM performance of sham-injured rats. The cognitive enhancing effects of Lu 25-109-T observed in this experiment are consistent with the findings of a previous study that also found that a 15-μmol/kg dose of Lu 25-109-T was effective in improving MWM performance of age-impaired rats (27,28). Lu 25-109-T readily crosses the BBB and reaches maximal neuronal bioavailability within 10 min of SC administration. Because Lu 25-109-T was administered 10 min prior to MWM assessment, it is assumed that maze learning occurred at a time when neuronal concentrations of Lu 25-109-T were elevated. Thus, it is not known whether the improvement in maze performance requires the pharmacological availability of Lu 25-109-T, or whether Lu 25-109-T promotes physiological recovery of some aspect of neuronal signaling. Thus, future investigations should test whether termination of chronic administration of Lu 25-109-T is also associated with improved cognitive performance. Nonetheless, the beneficial effects on MWM performance of Lu 25-109-T that were observed after experimental brain injury provide further support for long-term deficits in posttraumatic cholinergic neurotransmission. In addition, this study, along with other recent investigations (21,32,34), demonstrates that pharmacotherapeutic strategies designed to enhance cholinergic neurotransmission during the recovery period after TBI are suitable candidates for the treatment of TBI-induced cognitive dysfunction.

Past experience with cholinergic agonists for the treatment of senile dementia of the Alzheimer's type (SDAT) has proved disappointing. However, these early clinical trials employed relatively nonselective agonists such as arecoline, pilocarpine, or RS-86 (29). Presently, an increased awareness of the structure and function of mAChR subtypes, as well as the recent development of compounds that demonstrate a high selectivity for the subtypes of mAChRs, necessitates a reinvestigation of novel cholinergic therapeutics for the treatment of cognitive impairment affected by cholinergic hypofunction. For instance, central M_1 mAChRs are located postsynaptically, and stimulation of M_1 receptors causes the hydrolysis of phosphatidylinositol bisphosphate, which yields the intracellular second messengers inositol trisphosphate (IP_3) and diacylglycerol (41). In contrast, central M_2 receptors are located presynaptically and function as autoreceptors to control the release of ACh (12,41). Stimulation of M_2 autoreceptors results in a decreased activation of cAMP. Thus, the employment of nonselective mAChR agonists has opposing effects on cellular function, i.e., to stimulate postsynaptic M_1 receptors (desired effect) as well as to inhibit presynaptic release of ACh via stimulation of M_2 autoreceptors (undesired effect). Moreover, excessive nonselective stimulation of mAChRs has been shown to cause M_1 receptor downregulation (2,25,33) and to reduce ACh synthesis and release (1,4).

Because endogenous presynaptic release of ACh, and not the absolute concentration of synaptic transmitter levels, may be important to learning and memory processes, the equivocal results with nonselective agonists is not surprising. Thus, it has been suggested that a more physiologically appropriate strategy for cognitive enhancement would involve only the partial agonism of M_1 receptors and/or antagonism of presynaptic M_2 autoreceptors (29,35). Theoretically, partial agonism of the postsynaptic M_1 receptor would provide exogenous stimulation of understimulated M_1 receptors while preventing the receptor downregulation observed with full agonists. In addition, normal signaling between neurons is characterized by a complex and synchronous patterning of presynaptic transmitter release. Thus, antagonism of presynaptic M_2 autoreceptors would theoretically act to amplify the endogenous release of neurotransmitter in the synapse, and therefore would help maintain the complexity of interneuronal cell signaling.

Lu 25-109-T displays the profile of a partial muscarinic M_1 receptor agonist as well as that of a muscarinic M_2/M_3 receptor antagonist (27). Lu 25-109-T has a several-fold higher selectivity for the M_1 subtype than for the M_2 and M_3 subtypes ($\sim 9\times$ and $46\times$, respectively). In addition, receptor binding affinities of Lu 25-109-T for nicotinic, adrenergic α_1, α_2, and β; dopaminergic D_1 and D_2; serotonergic 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}, and 5-HT_3; histaminergic H_1; sigma σ_1 and σ_2; glutamatergic AMPA and NMDA; $GABA_A$; and benzodiazipine receptors indicate affinities more than 100–1000-fold lower than the muscarinic M_1 receptor binding affinity (27,28). Although M_1 mAChRs are almost exclusively neuronal, M_2 and M_3 mAChRs are located centrally and also have a widespread peripheral distribution, including localization in the heart, lung, and ileum (29). Thus, stimulation of peripheral M_2/M_3 mAChRs produces a host of unwanted side effects, including sweating, lacrimation, hypersalivation, hypertension, and tachycardia (29). Because of the M_2/M_3 antagonist effects of Lu 25-109-T, peripheral administration of Lu 25-109-T is not associated with the side effect liability profile of nonselective muscarinic agonists (27,28).

The pharmacological characterization (27,28) as well as the positive preclinical effects of Lu 25-109-T on cognitive function indicate that this compound may provide a promising alternative cholinomimetic therapy in cases of cholinergic hypofunction. This is in accord with other selective cholinomimetic therapeutics that have also been shown to be efficacious in improving cognitive performance after experimental TBI. For example, daily postinjury administration of Suritozole (MDL 26,479), an antagonist/negative-modulator of the γ-aminobutyric $acid_A$ ($GABA_A$) receptor, and BIBN 99, a selective M_2 mAChR antagonist, are both effective in attenuating cognitive deficits following central fluid percussion brain injury in rats (32,34). Thus, the recent synthesis of mAChR subtype-specific compounds offers a promising alternative for the treatment of disorders that affect cognitive functioning. However, it should also be emphasized that cognitive impairment following TBI, or other cognitive pathologies like SDAT, most certainly involve multiple neurotransmitter and other neurochemical alterations. Therefore, cholinergic enhancement is only one strategy by which recovery of cognitive function may be facilitated. In addition, because mAChRs have been found to be co-localized on noncholinergic neurons, e.g., M_2 heteroreceptors on glutamatergic neurons, the use of selective cholinomimetic drugs may also affect other transmitter systems. Thus, administration of selective cholinergic compounds may also facilitate transmission of other transmitter systems, e.g., NMDA, catecholamines, etc.

In conclusion, TBI results in a wide range of behavioral deficits, the most devastating of which involve cognitive dysfunction. The majority of pharmacotherapeutic strategies have been

focused on the immediate prevention or reduction of secondary injuries that occur as a result of mechanical impact to the brain. However, this strategy has a brief therapeutic window (≤30 min postinjury in rats) and has not produced very encouraging results in clinical trials. In addition, although prevalence rates are not known, there is currently a large population of head injury survivors that could potentially benefit from cholinomimetic pharmacotherapies. Further research into the complex mechanisms that maintain long-term cognitive deficits after brain trauma may provide many alternative pharmacotherapies for the treatment of cognitive dysfunction after TBI.

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