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Reduced Evoked Release of Acetylcholine in the Rodent Hippocampus Following Traumatic Brain Injury

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DIXON, C. E., J. BAO, D. A. LONG AND R. L. HAYES. *Reduced revoked release of acetylcholine in the rodent hippocampus following traumatic brain injury.* PHARMACOL BIOCHEM BEHAV 53(3) 679-686, 1996.—The chronic effects of traumatic brain injury on acetylcholine release were evaluated by using in vivo microdialysis. Acetylcholine release was measured in the hippocampus of anesthetized rats 2 weeks after lateral controlled cortical impact (n = 10) or sham surgery (n = 10). Prior to microdialysis, behavioral assessments of motor and spatial memory were performed. Cortical impact (6 meter/s, 2 mm deformation) produced beam balance deficits that persisted for 1 day and beam walking deficits that persisted for 3 days after injury. In addition, spatial memory, as measured by swim latencies in a Morris water maze, was compromised between 10–14 days after injury. Immediately following behavioral testing, the animals were anesthetized with halothane, and a microdialysis probe was placed into the dorsal hippocampus. After a 160 min equilibration period, extracellular levels of acetylcholine were measured prior to and after an intraperitoneal administration of scopolamine (1 mg/kg), which evokes acetylcholine release by blocking autoreceptors. Prior to scopolamine administration, there were no differences in extracellular levels of acetylcholine between injured and sham animals. However, there was a significant reduction of hippocampal acetylcholine release evoked by scopolamine in injured animals as compared to sham controls. In separate control groups, saline administration alone did not change hippocampal acetylcholine release in injured (n = 5) or sham (n = 5) animals. This study represents the first application of in vivo microdialysis to evaluate chronic neurotransmission deficits following TBI. The present study demonstrates that a magnitude of traumatic brain injury (TBI) sufficient to produce spatial memory deficits can result in a reduction in scopolamine-evoked release of acetylcholine within the hippocampus. The data further suggest that presynaptic mechanisms mediating release of acetylcholine could play a significant role in cholinergic neurotransmission deficits following TBI.

Brain injury Acetylcholine Microdialysis Scopolamine Rat

MEMORY deficits are among of the most persistent sequelae of traumatic brain injury (TBI) in humans (25). These memory disturbances include a period of transient posttraumatic amnesia characterized by gross confusion and an inability to commit new information to memory. After the resolution of posttraumatic amnesia, many patients exhibit persistent memory disturbances. Memory disturbances have been described as the most frequent complaint by patients and relatives 7 years posttrauma (34). While the neural basis for these deficits is unknown, chronic disturbances in cholinergic systems have been implicated in a number of neurodegenerative diseases that are associated with cognitive deficits (4,19,43,44). Moreover, cholinergic deficits have been implicated in Alzheimer's disease, and epidemiological studies have shown that TBI is a risk factor for Alzheimer's disease (31). Thus, chronic cognitive deficits following TBI may also be associated with altered cholinergic function.

Numerous laboratory studies have reliably demonstrated spatial memory deficits in rats ranging from 2 to 30 days following varying levels of TBI (9,12,18,26,39). Because these rodent studies assess functions that require an intact cholinergic system (3), the presence of deficits suggests that persistent

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memory disturbances following TBI in rats may be related to impaired cholinergic neurotransmission. Recent neurochemical studies have found alterations within cholinergic systems following TBI. High-affinity [^{3}H]choline uptake, a dynamic marker of cholinergic terminal function, has been found to be impaired 2 weeks following TBI (10). Also, TBI can significantly alter the binding sites of muscarinic receptors in the hippocampus and neocortex for as long as 15 days after TBI (21).

Recently, a scopolamine challenge paradigm has been used successfully in animals and humans to infer the status of central cholinergic functional integrity. Experimental TBI has been shown to produce a chronic enhanced sensitivity to the memory-disrupting effects of the muscarinic cholinergic antagonist, scopolamine (9,11). Patients with Alzheimers' disease, a syndrome associated with degeneration of the cholinergic system, exhibit a dose-dependent enhanced vulnerability to the memory disrupting effects of an acute administration of scopolamine (3,19,43,44). More recently, Alzheimer's patients have been shown to present increased pupil dilation response sensitivity to a muscarinic cholinergic blocker (38). These studies suggest that cholinergic integrity in the CNS can be assessed by measuring changes in responses normally sensitive to the effects of muscarinic receptor blockade. Moreover, mechanisms mediating evoked release of ACh by scopolamine have been extensively examined. Scopolamine can evoke ACh release by blocking presynaptic receptors, and, thus, preventing inhibition via negative feedback [see (41) for a review]. Thus, a scopolamine challenge paradigm is a useful and noninvasive tool for assessing alterations in central cholinergic transmission potentially contributing to the functional deficits following TBI.

Measurements of extracellular levels of ACh using brain microdialysis are sensitive to a variety of manipulations of the cholinergic system. For example, reduced extracellular ACh levels have been found in the hippocampus following lesions of the fimbria-fornix pathways (23,32). Also, brain microdialysis has been used to measure the reduction in spontaneous extracellular release of ACh in the cortex following mechanical and neurotoxic lesions to the basal forebrain (1). The microdialysis method is also sensitive to diurnal variations (28) and pharmacological manipulations of the cholinergic system with receptor agonists and antagonists (23,35,42,47). Thus, changes in extracellular levels of ACh, as measured by microdialysis, appear to be a sensitive marker of cholinergic function within specific brain regions.

Normal function of a cholinergic neuron is dependent upon its ability to release ACh. If the cholinergic deficits induced by TBI result from damage to cholinergic input to the hippocampus, this functional decline may be manifested by a decrease in the levels of evoked release of ACh in vivo. The first goal of the work presented here was to determine whether increased behavioral sensitivity to scopolamine following TBI (9) was attributable to altered release of ACh. Thus, we examined the effects of scopolamine-evoked release of extracellular ACh levels in the hippocampi of rats following TBI. This study represents the first application of in vivo microdialysis to evaluate chronic neurotransmission deficits following TBI. The second goal was to examine, in the same animals, the relationship between changes in ACh release and functional deficits by correlating extracellular ACh levels and Morris water maze performance. Morris water maze performance was selected as an outcome measure because it is sensitive to lesions of the hippocampus and cortex (29,30) and to manipulations of cholinergic function (9,45).

METHOD

Production of Experimental TBI

Injury device. The controlled cortical impact injury device (8) consisted of a small (1.975 cm) bore, double acting, stroke-constrained, pneumatic cylinder with a 5.0 cm stroke. The cylinder was rigidly mounted in a vertical position on a crossbar, which could be precisely adjusted in the vertical axis. The lower rod end had an impactor tip attached (i.e., part of the shaft that comes into contact with the exposed dura mater). The upper rod end was attached to the transducer core of a linear velocity displacement transducer (LVDT). The velocity of the impactor shaft was controlled by gas pressure. Impact velocity was measured directly by the LVDT (Shaevitz Model 500 HR), which produces an analog signal that was recorded by a PC-based data acquisition system (R. C. Electronics) for analysis of time/displacement parameters of the impactor.

Surgical procedures. All rats were anesthetized initially with 4% halothane with a 2 : 1 N_2O/O_2 mixture in a vented anesthesia chamber. Following endotracheal intubation, rats were ventilated mechanically with a 1–1.5% halothane mixture. Animals were mounted in the stereotaxic frame on the injury device in a supine position secured by ear and incisor bars. The head was held in a horizontal plane with respect to the interaural line. A midline incision was made, the soft tissues reflected, and two 7 mm craniotomies were made between lambda and bregma and centered 5 mm laterally on either side of the central suture. The animals received a cortical impact through the right craniotomy at a velocity of 6 meters/s. The injury device was set to produce a tissue deformation of 2 mm (n = 10). Sham rats (n = 10) underwent identical surgical procedures but were not injured. The sham rats were used to control for nonspecific methodological effects (such as those due to anesthesia and surgery). Core body temperature was monitored continuously by a rectal thermistor probe and maintained at 37–38°C. After injury, the scalp was sutured closed, and the animal extubated. Following recovery from anesthesia, animals were monitored visually and returned to cages where they received normal access to food and water.

Chronic Neurological Assessments

Motor function. Gross vestibulomotor function was assessed using a beam-balance task that consisted of placing the animal on a suspended, narrow wooden beam (1.5 cm wide) and measuring the duration it remained on the beam for up to 60 s. Training prior to injury consisted of three trials, during baseline measures (time on the beam) was measured. Finer components of vestibulomotor function and coordination were assessed using a beam-walking task (7,14). Also prior to injury, rats were trained to escape a bright light and loud white noise (Heath Model AD-1309) by traversing a narrow wooden beam (2.5 × 100.0 cm) to enter a darkened goal box at the opposite end of the beam. During training and testing, animals were placed at one end of the beam close to the source of light and noise. The noise and light were terminated when the rat entered the goal box at the opposite end of the beam. Four wooden pegs (3.0 mm diameter, 4.0 cm high) were placed in an alternating sequence along the beam to increase the difficulty of the task. Performance was assessed by measuring the animal's latency to traverse the beam. Data for each session consisted of the mean of three trials. The animal remained in the goal box for 30 seconds between trials.

Spatial memory. We employed a variant of the Morris water maze task that has been shown to be sensitive to cholinergic

function following TBI (9,11). This water maze task was used to compare rates of acquisition between injured and sham groups. Deficits in the ability to acquire new information are a common cognitive impairment following TBI in humans (37). The Morris water maze employed a pool 180 cm in diameter and 60 cm in depth. The pool was painted black and was filled with water to a depth of 28 cm. A clear Plexiglas platform 10 cm in diameter and 26 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., posters, pipes, bookcase) that remained constant throughout the experiment. Water maze testing started on day 10 postinjury to avoid the motor deficits observed following injury (18). Rats were given four trials per day for 5 consecutive days to establish baseline performance. For each daily block of four trials, rats were manually placed in the pool facing the wall. Rats started a trial once from each of the 4 possible start locations (north, east, south, west). The order of starting location was randomized. The goal platform was positioned 45 cm from the outside wall and was placed in either the northeast, southeast, southwest, or northwest quadrant of the maze. The location of the platform was held constant for each animal. Rats were given a maximum of 120 s to find the hidden platform. If the rat failed to find the platform within 120 s, it was placed on the platform by the experimenter. All rats were allowed to remain on the platform for 30 s before being placed in a heated incubator between trials. There was a 4-min intertrial interval. To confirm that differences in swim latencies reflect spatial memory deficits, a separate series of animals were either injured (n = 10) or sham-injured (n = 10) and a probe trial was employed to assess spatial memory following 5 days of hidden platform training. For the probe trial, the platform was removed and the percent time in the platform quadrant is measured by a video tracking system (San Diego Instruments Inc.) over a 60-s interval.

Microdialysis

Probe implantation. To minimize the interval between measurements of behavioral performance and ACh release, surgery for microdialysis began immediately following the last water maze assessment (2 weeks postinjury). The rats were initially anesthetized with 4% halothane with a 2 : 1 N_2O/O_2 mixture in a vented anesthesia chamber. Following endotracheal intubation, rats were ventilated mechanically with a 1–1.5% halothane mixture and their core body temperature was maintained at 37–38°C. Animals were mounted in a stereotaxic frame in a supine position secured by ear and incisor bars. A midline incision was made, the soft tissues reflected, and a small hole was made through the exposed dura to insert a concentric microdialysis probe (CMA/10, Carnegie Medicine) with a 2 mm-long dialysis membrane (0.5 mm in diameter) into the right dorsal hippocampus (A, 3.8; L, 3.5, V, −4.0 mm from bregma). The dialysate was perfused at a rate of 2.0 μl/min with artificial CSF (145 mM NaCl, 2.7 mM KCl, 1.2 mM $CaCl_2$, 1.0 mM $MgCl_2$, 2.0 mM Ca^3HPO_4 pH 7.4). To permit detection of ACh, the dialysis solution contained 5 μM neostigmine to inhibit acetylcholinesterase. Following a 160-min equilibration period (16), samples (40 μl total) were collected every 20 min and stored in ice. A total 40 μl of dialysate was collected to allow us to filter the sample prior to analyses and to leave us sufficient amount of sample to replicate the analyses if necessary. After samples were collected to establish basal levels of ACh, scopolamine (1 mg/kg) was injected IP and two more samples were collected. Two additional groups of animals underwent the same microdialysis procedure, but were injected with saline instead of scopolamine. At the end of the experiment, in vitro recovery rates for each probe were measured by placing the probe into a vial containing a 0.1 pmol/10 μl solution of acetylcholine and collecting for an additional 20 min. Finally, the animals were transcardially perfused with formalin and their brains removed to confirm histologically the placement of the probe.

HPLC instrumentation. ACh levels were measured in 10-μl from each sample using an isocratic HPLC-EC system. All equipment (including the guard column, analytical column and SPR) was obtained from ESA, Inc. (Bedford, MA) and consisted of a Model 580 pump with one pulse dampener, a Model 480 column oven and a Coulochem II Model 5200A electrochemical detector equipped with a Model 5040 solid state analytical cell, containing a PEEK/Pt target working electrode. Chromatograms were recorded and integrated on a PC-based data station which also provided remote RS-232 control of the detector.

Chromatographic and detector conditions. Using the chromatographic method of Greaney et al. (16), ACh was separated at a flow-rate of 0.35 ml/min on a polymeric reversed-phase column (ACH-3, 5 μm, 15 cm × 3 mm i.d.) equipped with an ACH-3-G guard cartridge. The mobile phase consisted of 100 mM sodium phosphate, 0.5 mM TMACl, 0.005% (v/v) Reagent MB and 2.0 mM OSA at a final pH of 8.0. ACh was enzymatically converted to hydrogen peroxide by a postcolumn SPR (containing immobilized acetylcholinesterase and choline oxidase) and was measured electrochemically on a Pt-target working electrode maintained at +300 mV (vs. Pd. reference). Consistent enzymatic efficiency and minimum changes in retention time were achieved by maintaining the column and SPR at 35°C. Pilot studies confirmed that the ACh levels reported below were within the detection limits of our HPLC and within a linear range of detection.

Histology

After the microdialysis experiments, the brains of the rats were examined histologically to confirm the placement of the microdialysis probe and to confirm qualitatively the morphologic extent of injury. As previously mentioned, rats were sacrificed by overdose of pentobarbital and transcardially perfused with 120 ml of saline followed by 120 ml of 10% formalin and their brains were postfixed in 30% sucrose for 2 days then frozen in dry ice. Coronal sections (20 μm) were cut at the level of the hippocampus, placed on microscope slide, and stained with hematoxylin and eosin for light microscopic evaluation.

Statistical Analysis

For each time point, data from the beam balance, beam walking, Morris water maze tasks, and microdialysis samples were examined by a separate repeated-measures (group × days) analysis of variance (ANOVA), where a significant effect was found in the ANOVA; individual group comparisons across days were made with a Tukey t post hoc test. For the probe trial, the mean percent time in the target quadrant was examined by Student's t-test. All data are expressed as mean ± standard error of the mean (SEM). A significance level of $p < 0.05$ was used for all tests.

RESULTS

Chronic Neurological Assessments

Figure 1A shows the latency of balancing on a beam on days 1–4 postinjury. Injured animals showed significant (in-

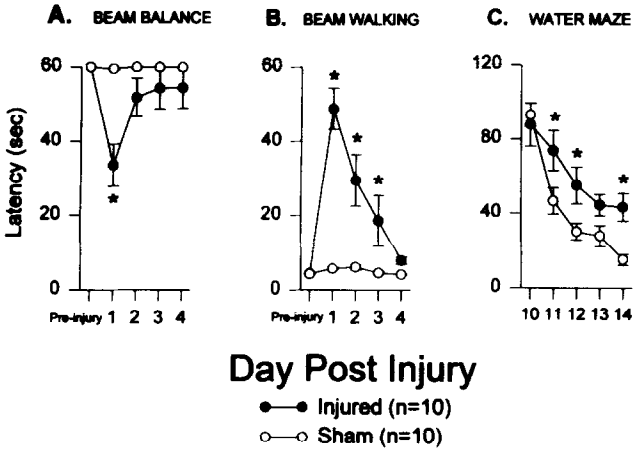

FIG. 1. (A) Beam balance deficits following lateral cortical impact injury. A graph plotting mean latency to balance on a beam across days postinjury. Prior to injury the animals were tested on their ability to balance up to 60 s on a beam. Beginning 1 day following injury, beam balance latencies were measured daily for 4 consecutive days. Injured animals showed a significantly shorter latency at 1 day postinjury than sham animals. (B) Beam-walking deficits following lateral cortical impact injury. A graph plotting mean latencies (± SEM) to traverse a beam across days postinjury. Prior to injury, animals were trained to traverse the beam within 5 s. Starting 1 day following injury, beam-walking latencies were measured daily for 4 consecutive days. Injured animals showed significantly longer latencies to traverse the beam on days 1–3 than sham-injured animals. (C) Water maze deficits following lateral cortical impact injury. A graph plotting mean latency (± SEM) to locate a hidden platform in the Morris water maze on days 10–14 postinjury. Injured animals showed significantly longer latencies to locate the platform than the sham animals. $*p < 0.05$.

jured = 33.5 ± 5.7 s vs. sham = 60 ± 0 s, $p < 0.01$) decreases in the balance durations on day 1 postinjury. However, after day 1 there was no significant difference in the latency between sham and injured animals. Figure 1B illustrates the latency to traverse the walking beam on days 1–4 postinjury. Injured animals showed significant (injured = 48 ± 5.6 s vs. sham = 5.6 ± 0.23 s, $p < 0.01$) increases in beam walking latencies on day 1. By day 4, the walking latencies of the injured group did not differ significantly from the sham group. Thus, both the beam balance and the beam-walking tests show that vestibulomotor deficits returned to preinjury levels by 4 days postinjury.

Water Maze Assessment

Figure 1C illustrates the latency to find the hidden platform over a 5-day training period beginning 10 days after injury. The mean latency for the four daily trials was calculated for each animal. The ANOVA indicated a significant group main effect on the latency to reach the hidden platform, $F(1, 19) = 6.87$, $p < 0.016$, indicating that the injured group has longer swim latencies than the sham group. During the probe trial (Fig. 2), injured rats spent significantly lower percentage of time, $t(18) = 2.97$, $p < 0.008$, in the target quadrant (35.76 ± 4.9%) than sham-injured animals (55.58 ± 4.5%). Chronic Morris water maze deficits have been hypothesized to be, at least in part, attributable to deficits in chronic cholinergic neurotransmission (3). We next examined indices of cholinergic neurotransmission: hippocampal ACh release pre- and postscopolamine administration in anesthetized animals.

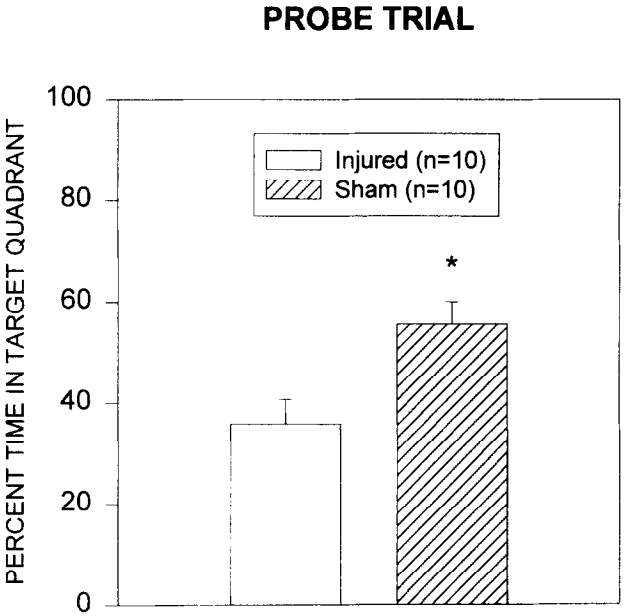

FIG. 2. Performance on the probe trial. The percent time spent in the training quadrant ± SEM, $*p < 0.05$.

Microdialysis

There were no significant differences in the in vitro recovery rates between the probes used for the injured group (12.2%) and the sham group (11.4%). In vivo results were not corrected with the in vitro recoveries because the addition of neostigmine decreases the reliability of estimates in in vivo recovery. Under halothane anesthesia, prescopolamine extra-

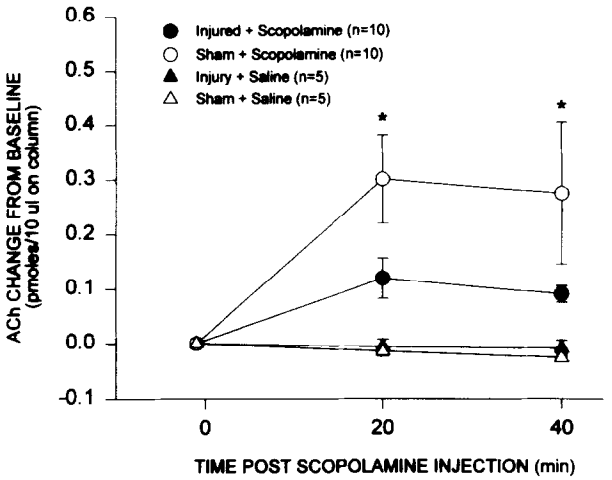

FIG. 3. The effects of scopolamine on extracellular levels of acetylcholine measured within the hippocampus two weeks after lateral cortical impact injury. A graph plotting mean percent change in ACh levels (± SEM) following scopolamine administration. Scopolamine evoked significantly less acetylcholine in injured hippocampi than noninjured hippocampi. Saline administration did not alter acetylcholine release in either injured or noninjured animals. Control administration of saline did not alter ACh release in either sham or injured rats. $*p < 0.05$.

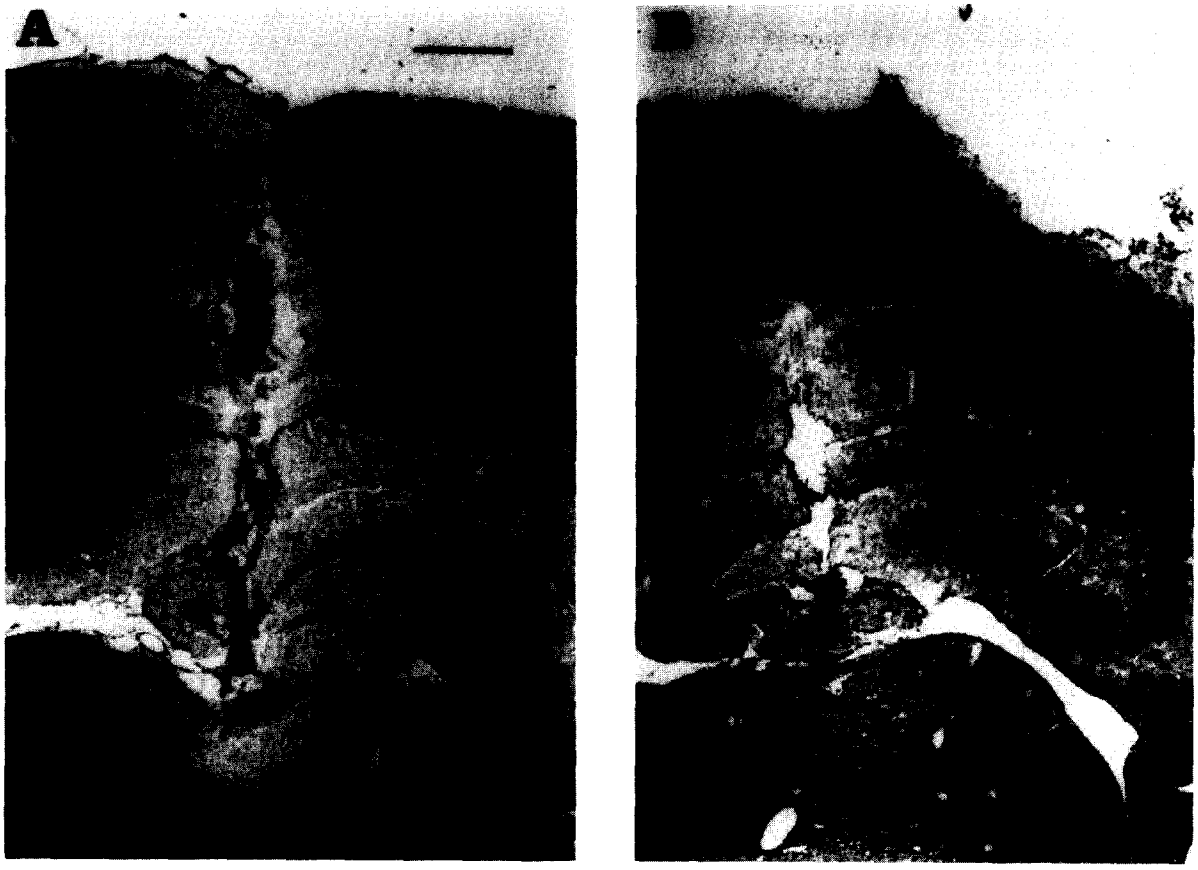

FIG. 4. H and E stained coronal sections through the dorsal hippocampus. (A) Photomicrograph showing the tract of the microdialysis probe yet otherwise unremarkable H and E coronal section from a sham animal. (B) Photomicrograph showing asymmetric bilateral cortical lesions at 2 weeks following injury. Hippocampus appears intact with no overt cellular or parenchymal pathology, except for the probe tract. Scale bar = 0.5 mm.

cellular levels of ACh within the hippocampus were not significantly different between injured (0.178 ± 0.020 (SEM) pmol/10 μl) and sham animals (0.174 ± 0.016 pmol/10 μl). However, the ANOVA indicated that following administration of scopolamine, injured animals released significantly less ACh than sham animals, $F(3, 39) = 7.48$, $p < 0.0008$. During the first 20 min after scopolamine administration, extracellular ACh levels in sham-injured animals increased 0.30 pmol/10 μl while levels in injured animals increased 0.12 pmol/10 μl (Fig. 3). Control administrations of saline did not alter ACh levels.

Histology

Light microscopic examination of serial hippocampal sections demonstrated that the microdialysis probes were within the CA1 region of the hippocampus of all rats. With the exception of the probe tract, representative hematoxylin and eosin sections from sham-injured rats appeared unremarkable (Fig. 4A). No evidence of hemorrhage, contusions, or overt cellular or neuropil damage was seen. In contrast, sections from injured brains demonstrated bilateral cortical lesions that were asymmetrical (Fig. 4B). Underlying subcortical structures such as the hippocampus appeared intact, with no overt cellular or parenchymal damage to the neuropil.

DISCUSSION

This study represents the first application of in vivo microdialysis to evaluate chronic neurotransmission deficits following TBI. The present study demonstrates that a magnitude of TBI sufficient to produce spatial memory deficits can result in a reduction in scopolamine-evoked release of ACh within the hippocampus. These data support the hypothesis that spatial memory deficits following TBI may be attributable, at least partially, to deficits in central cholinergic neurotransmission. More specifically, the present study suggests that presynaptic mechanisms play a significant role in post-TBI cholinergic neurotransmission deficits. As detailed below, possible changes in presynaptic function following TBI may include: a) reduced synthesis of ACh, b) altered autoreceptor binding and signal transduction affecting ACh release, and/or c) deafferentation of specific cholinergic pathways.

Reduced synthesis of ACh could contribute to persistent changes in cholinergic neurotransmission. A recent study of high affinity [^{3}H]choline uptake in the hippocampus of rats 2 weeks after cortical impact, measured in a synaptosomal preparation, found a reduction in the maximum velocity of choline uptake (V_{max}). No differences in affinity constants (K_m) were found (10). The results suggest that posttraumatic cholinergic deficits may be associated with a decreased ability

of cholinergic neurons to take up choline. Thus, scopolamine could evoke less ACh release if the rate of synthesis is compromised by reduced choline uptake. Studies directly measuring ACh synthesis are currently in progress in our laboratories.

Scopolamine may increase ACh release by blocking muscarinic autoreceptors whose normal function is to inhibit ACh release. A number of studies suggest that muscarinic autoreceptors are all of the M2 subtype (20,22,41). A recent examination of the muscarinic binding found decreased [^{3}H]-AFDX384 binding to the M2 receptor subtype in the hippocampal CA2-3 region and the dorsal blade of the dentate gyrus at 24 h following TBI (24). These reductions in receptor binding may persist for even longer periods and contribute to reduced scopolamine-evoked ACh release. However, it is important to recognize that, while in vitro experiments have shown that scopolamine can potentiate ACh release in hippocampal slices (46), in vivo experiments cannot definitively demonstrate that the site of action of scopolamine-evoked release is presynaptic. Thus, it is also possible that muscarinic antagonists may increase brain ACh release by blocking receptors located on the cell bodies of inhibitory interneurons (36).

The reduction in ACh release evoked by autoreceptor blockade following TBI may result from alterations in biochemical transduction pathways that link muscarinic presynaptic receptor stimulation to inhibition of calcium influx and/or changes in intracellular calcium levels to exocytosis of ACh containing vesicles. Recent evidence suggests that the blockade of autoreceptors increase ACh release by preventing inhibition of calcium influx through voltage-dependent calcium channels (33). However, if the inhibitory pathway between the autoreceptor and calcium channel is impaired, then basal release would be higher. Conversely, enhanced transduction between the autoreceptor and calcium channel may result in less release. Because basal levels between injured and sham animals did not differ, it is possible that autoreceptor regulatory function was not impaired. However, as discussed below, basal levels in this study could have been affected by a number of factors. Calcium can cause release by binding with a yet unknown molecule to trigger exocytosis (41). There is indirect evidence that TBI can alter intracellular calcium homeostasis. Following TBI, there are increases in total tissue calcium ion levels (40) and calcium accumulation (15) in the hippocampus that persist for several days. However, these studies did not measure calcium changes beyond 4 days, and measurements were not restricted to terminals where an increase in intracellular calcium may actually enhance scopolamine-evoked release.

Alterations in neurotransmission may also result from a loss of integrity of specific cholinergic pathways. While the integrity of hippocampal tracts has not been specifically examined in the cortical impact model, a recent study examining cholinergic neurons in the medial septal nucleus and the diagonal band of Broca 10 days after fluid percussion TBI showed significant decreases in choline acetyltransferase positive neurons (24). Thus, it is possible that scopolamine evokes less ACh in injured animals because of cholinergic terminal loss associated with deafferentation of the septal-hippocampal pathway. Our laboratory is currently conducting immunohistologic studies to evaluate more precisely the integrity of cholinergic pathways in the rodent cortical impact model.

Prior to scopolamine administration, no differences in basal extracellular ACh levels were observed between injured and sham control animals, although there were significant differences in water maze performance deficits. The failure of TBI to alter basal ACh levels may be attributable to a number of features of our experimental procedures, including the use of neostigmine in the perfusion medium and/or our anesthetic regimen. Due to the low basal output of acetylcholine in the hippocampus, it is necessary to increase the output of acetylcholine by adding an acetylcholinesterase inhibitor to the perfusion medium (5). Increasing ACh levels with neostigmine may mask small changes in basal cholinergic neurotransmission. In addition, halothane, the anesthetic used in the present study, can produce a generalized decrease in ACh release (6). Anesthetic effects can be avoided by performing microdialysis in awake, freely moving animals (28). We have completed preliminary studies evaluating the effects of TBI on scopolamine evoked release in awake behaving animals (13). These preliminary studies replicated the present data, though at higher ACh levels. Therefore, while anesthetics can suppress overall ACh release, the primary observation that injured rats release less ACh in response to scopolamine than sham-injured rats is not likely an artifact of conducting an acute experiment.

Although injured animals had deficits in both spatial memory and hippocampal scopolamine evoked ACh release, these data do not prove that hippocampal cholinergic neurotranmission deficits cause spatial memory deficits following TBI. It is also possible that alterations in scopolamine-evoked release measured in the hippocampi of anesthetized animals and spatial memory performance are not related. There are conflicting data whether the hidden platform water maze task is sensitive to cholinergic denervation of the hippocampus. A recent study found that cortical, but not hippocampal denervation was sufficient to impair spatial memory in the Morris water maze (2). However, deficits in spatial learning tasks have been reported following electrolytic and excitotoxic lesions of the medial septal area (17,27). Additonal research is needed to more closely examine the relative contributions of hippocampal and cortical cholinergic neurotranmission deficits to spatial memory dysfunction following TBI.

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