BRIEF COMMUNICATION

Cannabinoid agonist rescues learning and memory after a traumatic brain injury

Marium Arain[1], Maida Khan[1], Laura Craig[2] & Stan T. Nakanishi[1,3]

[1]Alberta Children's Hospital Research Institute, University of Calgary, Calgary, Alberta, Canada
[2]Regeneration Unit in Neurobiology Core Facility, University of Calgary, Calgary, Alberta, Canada
[3]Department of Biology, University of Hawai'i at Hilo, Hilo, Hawai'i, USA

Correspondence
Stan T. Nakanishi, Department of Biology, University of Hawai'i at Hilo, Hilo, Hawai'i, 96720, USA. Tel: 808-932-7193; Fax: 808-932-7295; E-mail: stn@hawaii.edu

Abstract

Traumatic brain injury can cause persistent challenges including problems with learning and memory. Previous studies suggest that the activation of the cannabinoid 1 receptor after a traumatic brain injury could be beneficial. We tested the hypothesis that posttraumatic brain injury administration of a cannabinoid 1 receptor agonist can rescue deficits in learning and memory. Young adult male rats were subjected to a moderately severe controlled cortical impact brain injury, with a subset given postinjury i.p. injections of a cannabinoid receptor agonist. Utilizing novel object recognition and the morris water task, we found that the brain-injured animals treated with the agonist showed a marked recovery.

Introduction

Traumatic brain injury (TBI) occurs when brain tissue is damaged by a force applied to the head, and affects an estimated 1.7 million people per year in North America.[1] TBI patients often contend with persistent challenges including problems with learning and memory.[2] Currently, there are no effective treatments for post-TBI cognitive deficits, but research has uncovered some of the pathophysiological mechanisms which include: excitotoxicity, neuroinflammation, and neurometabolic dysfunctions with an associated increase in reactive oxygen species.[3]

The endocannabinoid system's ligands and receptors are broadly distributed throughout the body and have diverse modulatory functions.[4] In the brain, the cannabinoid receptor 1 (CB1R) mediates the rewarding aspects of marijuana,[5] and has been shown to decrease excitotoxicity,[6] suppress neuroinflammation,[7] and modify neurometabolism.[8] These studies suggest that administration of a CB1R agonist after a TBI could prevent or reduce the injury-associated cognitive deficits.[9] Clinical and animal studies provide clear evidence for learning and memory deficits in many forms and models of TBI. Other studies provide strong support for the potential therapeutic potential of modulating cannabinoid signaling to improve outcomes after a TBI,[10,11] but there is limited information about the behavioral outcomes of applying cannabinoid receptor agonists to address post-TBI deficits in learning and memory. We tested the hypothesis that administration of a CB1R agonist after a TBI would rescue learning and memory-dependent behaviors in young adult male rats.

Methods

Animals and treatment groups

All procedures were approved by the University of Calgary's Health Sciences Animal Care Committee, Calgary, Canada. The study was performed using male Sprague–Dawley rats ($n = 33$, 70–75 days old, ~300–360 g, Charles River, St. Constant, Canada), housed in pairs, with 12/12 light/dark cycle, and standard rat chow and water provided ad libitum. Rats were randomly assigned to one of five groups: TBI + drug ($n = 8$), TBI + vehicle ($n = 8$), sham + drug ($n = 6$), sham + vehicle ($n = 7$), or naïve

($n = 4$, Fig. 1A), and housed with an identically treated cage mate. The time course of the controlled cortical impact (CCI) surgery, drug/vehicle injections, battery of behavioral tests, euthanasia, and brain tissue extraction is shown in Figure 1B.

Controlled cortical impact

The TBI treatment groups were subjected to a moderately severe CCI as described by others.[12] Briefly, the rats were anesthetized with Isoflurane (Pharmaceutical Partners of Canada, Inc., Richmond Hill, Canada), head secured in a stereotaxic frame, and a craniotomy was performed. The CCI piston struck the brain tissue at 4.0 m/sec, with a 2.5 mm depth, for 100 msec (Precision Systems and Instrumentation, Lexington, Kentucky USA). The sham treatment groups were subjected to the same procedure, minus the impact. Following surgery, the wound was closed and animals were transferred to a warm cage for recovery. Naïve animals received no surgical treatments.

Drug and vehicle injections

Rat assigned to the TBI + drug and sham + drug treatment groups were administered the CB1R agonist arachidonyl-2′-chloroethylamide (ACEA, 1 mg/kg, daily, i.p., Cayman Chemicals, Ann Arbor, MI) dissolved in dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO),

Tween80 (polyethylene sorbitol ester, Sigma-Aldrich, St. Louis, MO), and sterile saline 0.9% in a 1:1:18 proportion, with a final injection volume of 1 mL/kg. Rats assigned to the TBI + vehicle and sham + vehicle groups received identical injections with the exception of the ACEA component. Rats in the TBI + drug, sham + drug, TBI + vehicle, and sham + vehicle groups received their first injection within 5–10 min after their respective surgical procedure, and received daily injections for the next six consecutive days. Rats assigned to the naïve treatment group were not subjected to any surgical procedures or injections.

Behavioral tests and histology

Behavioral tests were used to evaluate all the animals in this study. The order of the tests progressed from the putatively least to more stressful[13]: open field (OF), elevated plus maze (EPM), novel object recognition (NOR), and finally the morris water task (MWT). For the OF test, rats were placed individually in a 90 × 90 × 40 cm square container for 10 min and their movements were automatically tracked (SMART 3.0; Panlab/Harvard Apparatus, Barcelona, Spain).

For the EPM test, rats were placed in the center of a four-armed structure, with two open and two closed arms (60 cm long × 20 cm wide; elevated 60 cm off the ground) for 10 min; their behavior was video recorded for offline analysis. For the NOR, rats were individually habituated in a chamber (50 × 50 × 50 cm) for 10 min each for 3 days, were placed in the chamber with two identical small items for a 5-min learning phase, removed for either 15 min or 24 h (counterbalanced for objects, object location, time of day, and test interval), and then placed back in the chamber with one familiar item and one novel item for 5 min, during which the proportion of time spent with the novel item was divided by the total time investigating either object in the chamber.[14] For the MWT, rats were given eight daily trials (the starting point for each trial was randomly selected from four cardinal positions, each used twice), for five consecutive days, in a 1.8-m-diameter water-filled pool containing a submerged escape platform in one quadrant of the pool. On day 6, the probe trial (60 sec) was conducted with the escape platform removed.[15] Throughout the MWT testing, each rat's behavior was video recorded and quantified online (ViewPoint, Lyon, France).

After completing the behavioral tests, the animals were sacrificed, and their brains were processed for histological analysis. Brains were sectioned at 25 μm using a cryostat, stained with cresyl violet, and imaged. Lesion volumes were calculated using ImageJ (U.S. National Institutes of Health, Bethesda, Maryland, USA).
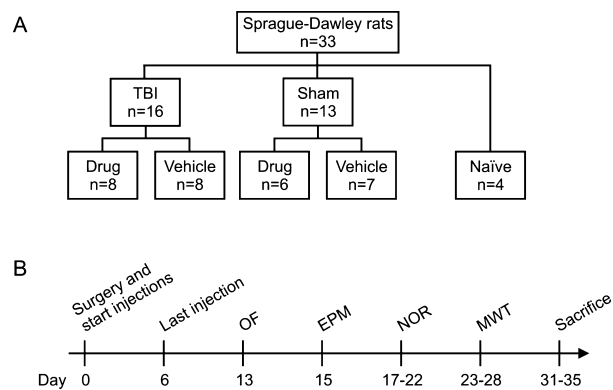

Figure 1. Study design and time course. (A) Diagram of the treatment groups and number of animals within each group (*n*). (B) Time course of surgical treatments, injections, behavioral testing, and animal sacrifice. The time course of the study started on Day 0, at which the rats were 70–75 days old and progresses through the injection treatments completing on Day 6, followed by behavioral testing: open field (OF), elevated plus maze (EPM), novel object recognition (NOR), and morris water task (MWT). The study was concluded between Days 31–35 when the animals were sacrificed.

Statistical tests

All reported values are presented as mean ± standard error of measurement (SEM), with analysis of variance (ANOVA) (one-way) and Tukey's post hoc tests used to evaluate significant differences between groups unless otherwise specified, with $P < 0.01$ interpreted as a significant difference. Statistical tests were executed using GraphPad Prism (GraphPad Prism for Windows, GraphPad Software, La Jolla, CA).

Results

Body mass, and OF and EPM behaviors

Throughout the study, there were no significant differences in the body mass measurements recorded immediately before (330.2 ± 2.4 g, $P = 0.93$) and after surgery (328.0 ± 2.4 g, $P = 0.38$), at the end of the drug treatment phase (344.4 ± 3.2 g, $P = 0.42$), or at the end of the study when the animals were euthanized (389.6 ± 3.7 g, $P = 0.26$). There were no differences between the treatment groups in their OF behaviors including total distance travelled (5832 ± 208.3 cm, $P = 0.54$), percent time in outer area (91.96 ± 0.89%, $P = 0.38$), or average velocity (9.72 ± 0.35 cm/sec, $P = 0.54$); suggesting that there were no locomotor deficits associated with the treatments.[16] Similarly, there were no differences in the set of quantified EPM behaviors: time in closed arms (488.2 ± 11.6 sec, $P = 0.83$), closed arm entries (11.6 ± 1.0 entries, $P = 0.40$), time in center (70.1 ± 7.5 sec, $P = 0.71$), percent open entries/total entries (20.3 ± 1.9%, $P = 0.44$), percent time in open arms (6.9 ± 1.2%, $P = 0.49$), and the total number of entries (14.7 ± 1.3, $P = 0.40$). The EPM results suggest that there were no differences in locomotor or anxiety-associated behaviors between the groups.[17]

Novel object recognition

At the short time interval (15 min), rats in the TBI + vehicle group showed a statistically significant deficit in their discriminatory index (0.54 ± 0.07), while TBI + drug animals showed a discriminatory index (0.79 ± 0.05) that was indistinguishable from the sham + drug, sham + vehicle and naïve groups, respectively (0.85 ± 0.03, 0.82 ± 0.05, 0.78 ± 0.1, $P < 0.01$, Fig. 2A). However, there were no significant difference between any of the groups at the 24-h test interval ($P = 0.21$, Fig. 2B).

Morris water task

On day 1 of the acquisition phase, there was no significant difference in latency to the platform between the treatment groups (37.6 ± 1.5 sec, $P = 0.45$). By day 2, the TBI + vehicle group latency to the platform (43.7 ± 5.4 sec) was significantly longer than the other groups ($P < 0.001$), which did not differ from one another (TBI + drug: 24.5 ± 3.9 sec, sham + drug: 16.9 ± 2.7 sec; sham + vehicle: 21.9 ± 3.9 sec, and naïve: 14.3 ± 3.4 sec). The significant differences between the TBI + vehicle group and the other treatment groups, which did not differ from each other, continued through acquisition day 3 ($P < 0.001$), day 4 ($P < 0.0001$), and day 5 ($P < 0.0001$, Fig. 2C). Furthermore, there was no significant difference in the latency time between day 1 (40.0 ± 2.8 sec) and day 5 (35.9 ± 3.4 sec) for the TBI + vehicle group (paired *t*-test, $P = 0.23$). On day 6, the escape platform was removed and each rat was given a single 60-sec probe trial where there was a significant difference in the percent of time spent in the target quadrant ($P < 0.0001$); the TBI + vehicle group spent less time in the target area (30.8 ± 5.7%) than any of the other treatment groups (TBI + drug (53.6 ± 4.5%), sham + drug (56.3 ± 5.3%), sham + vehicle (72.0 ± 3.5), and naïve (77.1 ± 3.4), Fig. 2D). Throughout the MWT study, there were no differences in swim speeds between the treatment groups on any given day ($P = 0.89$).

Lesion volume

There were no measurable brain lesions in animals in the sham + vehicle, sham + drug, or naïve treatment groups. In contrast, prominent lesions were observed in the TBI + drug and TBI + vehicle treatment groups (Fig. 3A). There was no statistically significant difference in the calculated lesion volumes between TBI + drug (4.84 ± 1.08 mm^3) and TBI + vehicle (3.44 ± 0.78 mm^3, $P = 0.31$, Fig. 3B) treated animals. Similarly, there was no significant difference in the percent lesion volumes between TBI + drug (7.45 ± 1.31%) and TBI + vehicle (6.42 ± 1.57%, $P = 0.62$, not shown).

Discussion

This study demonstrated that the administration of a CB1R agonist (ACEA) after a moderately severe experimental TBI rescued learning and memory abilities in young adult male rats. We found no differences in general locomotor coordination or anxiety-associated behaviors using the OF or EPM. For NOR, we found that the CB1R agonist treatment rescued the post-TBI deficit at the short time interval (15 min), and that the drug treatment also fully rescued spatial memory in the MWT. Taken together, this study shows that the administration of a CB1R agonist after a TBI rescues deficits in learning and memory.
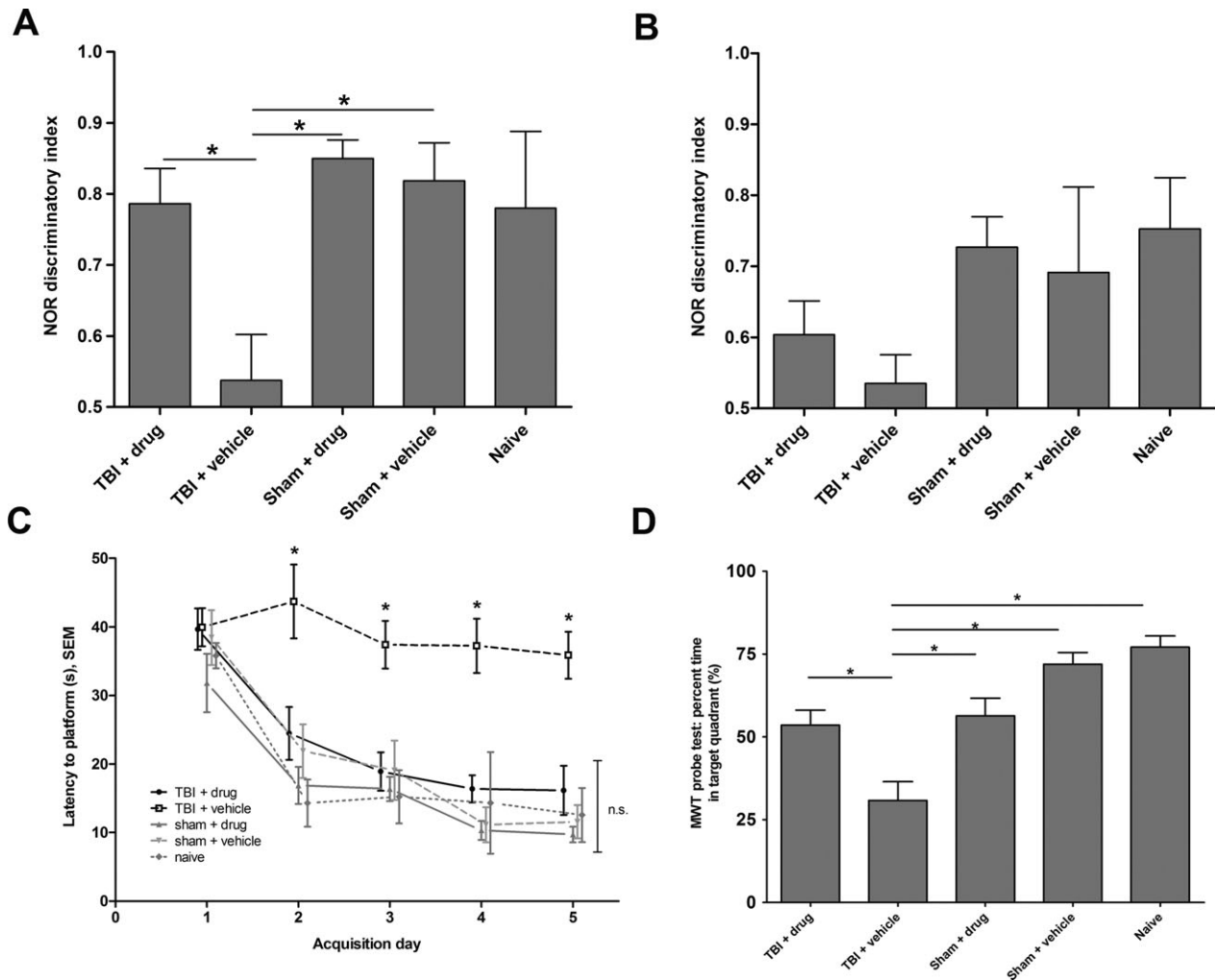
Figure 2. Administration of a CB1R agonist (ACEA) rescued learning and memory-associated behaviors. (A) NOR test at the short time interval (15 min) showed that the TBI + vehicle treatment group had a significantly lower discriminatory index compared to the other treatment groups. Notably, the TBI + drug group was indistinguishable from the sham and naïve groups. (B) There were no significant differences between the treatment groups for the NOR test at the long time interval (24 h). (C) During the acquisition phase of the MWT, the TBI + vehicle treatment group took significantly longer to find the escape platform over the course of 5 days. In contrast, the TBI + drug and other treatment groups did not differ from one another. (D) The single probe trial following the MWT acquisition phase showed that the TBI + vehicle group spent significantly less time in the target quadrant than the other treatment groups. CB1R, cannabinoid receptor 1; ACEA, arachidonyl-2′-chloroethylamide; NOR, novel object recognition; TBI, traumatic brain injury; MWT, morris water task.

If there was no difference in the sizes of the lesions, why did not the TBI + drug animals show behavioral deficits like the TBI + vehicle animals? Although the CCI injury is a focal TBI with mechanical damage highly localized to the impact area, aspects of the pathological signaling cascades associated with excitotoxicity, neuroinflammation, and metabolic dysfunctions may be dispersed both ipsilateral and contralateral to the directly affected hemisphere.[18,19] We speculate that for this current study, the cannabinoid agonist treatment may have preserved learning and memory in the TBI-treated animals by protecting the intact brain tissue that was not directly damaged by the primary injury, and that the "rescued" brain areas were then able to compensate for the lesioned areas.[20] Alternatively, and/or in parallel, the cannabinoid receptor agonist treatment could also have limited cerebral edema and neuronal cell loss,[10,11] diffuse axonal damage, decreased pathological neuroinflammatory processes, or modulated metabolic processes that preserved neuronal tissues or functions.[21] Future experiments could investigate and differentiate these potential mechanisms and identify the most likely sites of the cannabinoid receptor agonist efficacy.
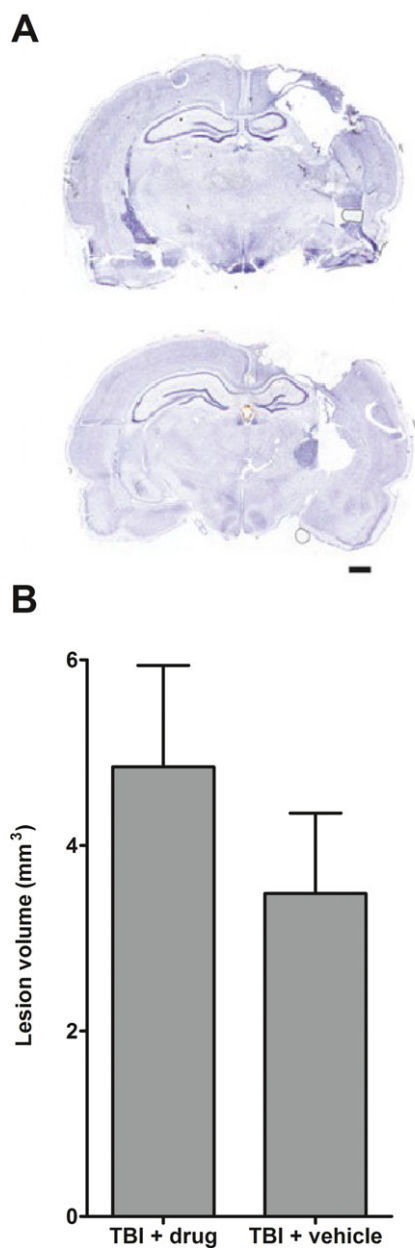
Figure 3. Cannabinoid receptor agonist treatment did not affect the traumatic brain injury (TBI) lesion volume. (A) Representative cresyl violet-stained coronal brain sections showing brain tissue damage to the right hemisphere. (B) There was no significant difference in the lesion volume between TBI + drug and TBI + vehicle treatment groups.

In this study, we aimed to use the lowest dose of the cannabinoid agonist, ACEA (1 mg/kg) that might be efficacious, based on previous studies.[22,23] The reason we selected a low dose are several fold; to minimize nonspecific pharmacological effects, to limit potential intoxication-induced behavioral changes, to evade the possible seizure-genic effects shown with prolonged or high doses

of cannabinoid agonists,[24,25] and lastly, that a low-dose cannabinoid treatment might potentially be more palatable for potential clinical studies in the future.

Acknowledgments

Conflict of Interest

None declared.

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