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Subchronic cerebrolysin treatment alleviates cognitive impairments and dendritic arborization alterations of granular neurons in the hippocampal dentate gyrus of rats with temporal lobe epilepsy

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ABSTRACT

Temporal lobe epilepsy (TLE) is one of the most frequent forms of focal epilepsy; patients with this condition, in addition to exhibiting complex seizures, also exhibit cognitive deficits. In the temporal lobe, the hippocampus, a structure relevant to learning and memory processes, is particularly affected by epilepsy. In animal models of TLE induced by pilocarpine, learning and memory deficiencies associated with changes in synaptic plasticity of the hippocampus have been reported. Cerebrolysin (CBL) is a biologically active mixture of low molecular weight peptides with neuroprotective and neurotrophic effects. The objective of the present study was to determine whether subchronic CBL treatment of rats in the chronic phase of TLE reduces the number and intensity of seizures, and whether CBL treatment can improve cognitive deficits (learning and spatial memory) and dendritic morphology in granular dentate neurons of the hippocampus. Temporal lobe epilepsy (lithium–pilocarpine model) was induced in male Wistar rats (weight, 250–300 g). Two epileptic groups were studied, in which CBL (538 mg/kg) or vehicle was administered intraperitoneally for 5 consecutive days per week for 3 weeks. Respective controls were also included in the study. At the end of treatment, the Barnes maze test (BMT) was used to assess spatial navigational learning and memory. The dendritic morphology of the dentate gyrus was also evaluated using the Golgi–Cox staining method. Results of this study did not support an antiepileptic effect of CBL. Epileptic animals treated with this agent exhibited secondarily generalized seizures similar in frequency and intensity to those of epileptic animals treated only with vehicle. However, when analyzing dendritic morphology of hippocampal granular neurons in these animals, CBL appeared to attenuate dendritic deterioration caused by epilepsy, which was associated with improved cognitive performance of the CBL-treated animals in the BMT compared with vehicle-treated epileptic rats. In conclusion, although CBL did not exert an anticonvulsant effect against secondarily generalized seizures, it can be proposed for use as an add-on therapy in epilepsy management to prevent neuronal alterations, and to improve memory and learning processes.

1. Introduction

Epilepsy is an alteration of the central nervous system characterized by abnormal increases and synchronization of neuronal electrical activity, which manifest as recurrent and spontaneous seizures, as well as electroencephalographic changes [1]. Temporal lobe epilepsy (TLE) is the most common form of epilepsy, with focal seizures originating in one of the temporal lobes [2]. In 80% of cases, the epileptogenic focus originates in hippocampus, which is the most excitable region of the human brain; this epileptogenic focus is frequently associated with pathological alterations in the hippocampus [3].

Most characteristics of TLE can be reproduced in chronic animal models, for example, those induced by pilocarpine, which replicate the natural development of symptomatic focal epilepsy that results from an initial insult, such as status epilepticus (SE) [4]. This model is characterized by structural, biochemical, and behavioral changes that occur in 3 consecutive periods beginning with SE [5]. Subsequently, the latent period (epileptogenesis) occurs, in which morphological and functional changes are generated [6]. Finally, after days or weeks, the chronic period begins, which is characterized by the presence of spontaneous recurrent seizures (SRS) [5,7].

Studies involving patients [8,9] and animals [10] with TLE suggest that cognitive impairments are a common comorbidity, which may even persist with sufficient control of seizures. The mechanism by which TLE pathology induces cognitive impairment has not been firmly established; however, it may involve severe neuronal cell loss and gliosis [5], as well as changes in synaptic hippocampal plasticity, for

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example, mossy fiber sprouting, interneuron loss, and ectopic dentate granule cell proliferation [5,11,12]. Here, the dentate gyrus (DG), the input region of the hippocampus, plays a critical role in learning, memory, and spatial coding processes [13]. Several lines of evidence suggest that granular cells (the main cell type in the DG) act as a preprocessor of incoming information, preparing it for subsequent processing in pyramidal cells of the CA3 region [14]. Recently, it has been proposed that cognitive impairment in TLE is also the result of neuronal network disruption [8]. These changes in neuronal and network properties provide a strong rationale for the development of neuroprotective treatments to prevent the adverse long-term consequences of seizures.

Cerebrolysin (CBL) is a biologically active mixture of low molecular weight peptides with neuroprotective and neurotrophic effects [15,16]. It has been proposed that the beneficial effects of CBL treatment are related, in part, to its ability to mimic the effect of endogenous neurotrophic agents (reviewed in [17]). Additionally, CBL administration may facilitate restoration of dendritic length and dendritic spine density in the limbic regions of aged mice [18,19]. Cerebrolysin also improves synaptic and dendritic pathology in animal models of schizophrenia [20,21]. These may be some of the reasons why CBL has been used in the treatment of neurodegenerative diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease, dementia, and acute or chronic stroke [17,22,23]. Moreover, CBL is also used in the treatment of neurodevelopmental disorders including autism and schizophrenia (reviewed in [24]) [25]. However, the limited number of studies investigating CBL in epilepsy has yielded varied results. For example, in a recent study, Gromova et al. [26] found that CBL treatment reduced the severity and duration of seizures caused by thiosemicarbazide (a primary generalized seizure model) and increased the survival rate of rats. In contrast, in humans, approximately 9% of patients with severe disability after traumatic brain injury exhibited seizures after treatment with CBL [27]. Because of this apparent proconvulsive effect of CBL, subjects with epilepsy were excluded from studies investigating the efficacy of CBL in infants with perinatal brain insult [25].

In contrast, a group of agents known as antiepileptic drugs (AEDs) is used to control epileptic seizures. However, in some cases, AEDs do not improve cognitive impairments and synaptic reorganization, which are a common comorbidity in TLE [8,10]. Therefore, studies investigating new medicines for unique or add-on therapies in the management of epilepsy and its comorbidities are needed. Accordingly, the objective of the present study was to identify whether CBL treatment during the chronic period of TLE reduces SRS and ameliorates cognitive impairments by conserving the dendritic morphology of granular cells in the hippocampus of epileptic rats.

2. Materials and methods

Thirty male Wistar rats (weight, 250–300 g), housed under standard conditions (regulated temperature [22 ± 2 °C]; light/dark cycle [12:12 h (lights on 07:00)]) and ad libitum access to food and water, were used in the present study. The rats were randomly allocated into one of four groups: control (CONT [n = 7]), epileptic (EPI [n = 8]), CONT-CBL (n = 7), and EPI-CBL (n = 8). All experimental procedures were conducted in accordance with Mexican law (SAGARPA NOM-062-Z00-1999) and the National Institutes of Health, Guide for the Care and Use of Laboratory Animals. The following experimental protocol was used (Fig. 1). In the epileptic groups (i.e., EPI and EPI-CBL), SE (see Section 2.1) was induced at week 0.

In turn, control rats (i.e., CONT and CONT-CBL) were maintained in similar conditions, except that SE in these animals was not induced. Two weeks after SE induction, the rats were treated with CBL or vehicle for three weeks. At the end of treatment, the Barnes maze test (BMT) was conducted for one week, and one day thereafter, intracardial perfusions were performed. The brains of the animals were then removed and processed for histological analysis. The rats were video monitored for five weeks (one week before starting the treatments through to the end of the study) to record SRS.

2.1. Induction of TLE

To induce TLE in the rats (modified from [28]), the rats were pretreated with lithium chloride (127 mg/kg [intraperitoneal (ip)], Sigma, México) 21 h before pilocarpine administration. On the day of SE induction, the animals were injected with scopolamine methyl-bromide (1 mg/kg ip, Sigma, México), and 30 min later, they received a single dose of pilocarpine hydrochloride (60 mg/kg ip; Sigma, México). Behavioral seizure classes were scored according to the Racine scale [29] to determine the onset of SE, which was defined as sustained convulsive behavior (stage 4 or 5 on the Racine scale [see Section 2.2]) for > 30 min. Rats that did not exhibit behavioral changes or scored <4 on the Racine scale were removed from the study [30].

Ninety minutes after SE induction, the rats received an intramuscular injection of diazepam 5 mg/kg and were placed on an ice bed for 1 h to mitigate the hyperthermia produced by SE. A second dose of diazepam was administered 8 h later, and finally, the rats were housed overnight in a room at 17 ± 2 °C. Two days after SE induction, the room temperature was restored to 22 ± 2 °C.

2.2. Monitoring of spontaneous recurrent seizures

One week after SE induction, the rats were allocated to individual acrylic cages and were continuously (24 h/7 days) video monitored using a 4-camera system (EV1004TURBOX, EPCOM, México City) for 5 weeks. The videos were analyzed by trained observers (blinded to the treatments) to detect behavioral seizures using the fast-forward (8×) feature of the system. When seizure-like activity was detected, the video was reversed to the start of the behavior and examined at real-time speed. An animal was considered to exhibit a convulsive seizure when the Racine score reached ≥4 [29]. Convulsive seizure activity was reflected by bilateral forelimb clonus, with rearing and falling (stage 4), and generalized tonic–clonic activity with loss of postural tone, often resulting in wild jumping (stage 5). With these criteria, SRS were considered as secondarily generalized seizures, as suggested by Goffin et al. [31]; in this classification, stages 1–3 were referred to as partial seizures. Datasets were generated recording the latency to the first stage 4 or 5 seizure, the number of seizures per week (SRS frequency), and seizure duration, which was measured from the onset of stage 4 seizure until stage 4 or 5 seizure termination.

2.3. CBL treatment

Two weeks after SE induction (epileptogenesis period), CBL (538 mg/kg; Renacenz, Ever Neuro Pharma GmbH, Unterach, Austria) was intraperitoneally administered daily for 5 consecutive days followed by 2 days without treatment per week, for 3 weeks. The CBL dose was chosen based on previous experiments involving epileptic rats [26].

2.4. BMT

The BMT has been used to assess spatial navigational learning and memory in a wide range of rodent models [32]. Three days after completing the treatments, animals from all groups were subjected to the BMT. Briefly, the BMT apparatus consists of a circular platform, 120 cm in diameter and 115 cm in height. There are 18 holes with 10 cm diameters cut in the platform that are equidistantly spaced and arranged along the perimeter, only one of which leads to the refuge cage (35 × 16 × 20 cm). The remaining holes have false bottoms covering the nonescape holes preventing the animal from falling out. Clues outside of the maze were placed on the walls of the test room, and a video camera fixed above the Barnes maze was used to acquire data. For each animal, the position of the refuge cage was the same for all trials. The test

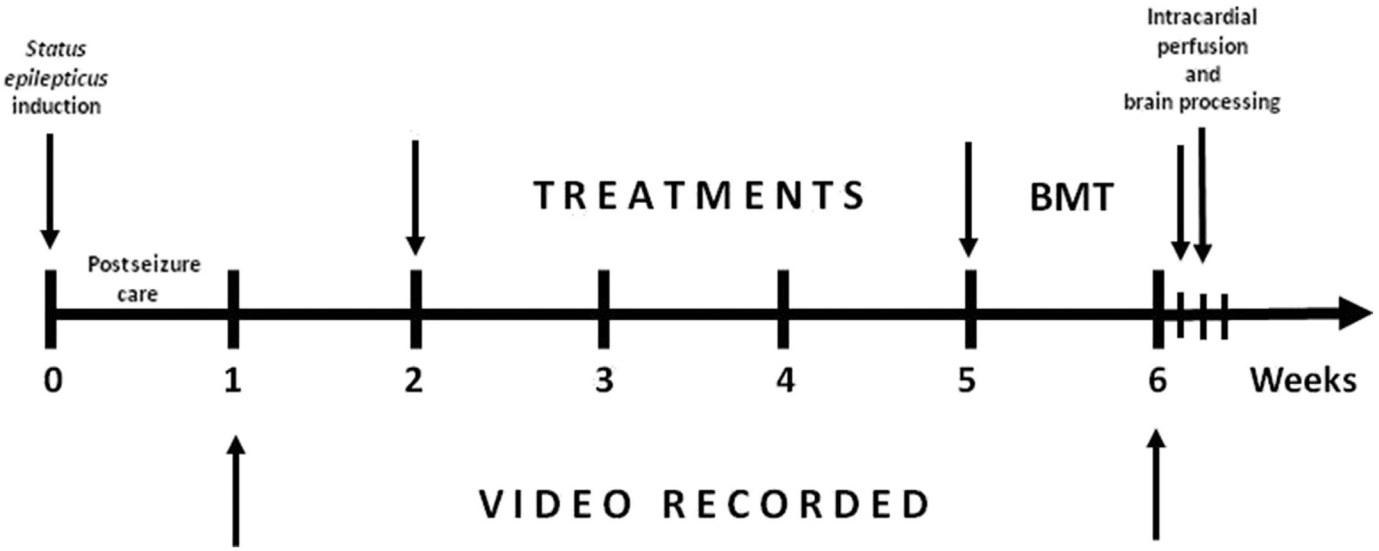

Fig. 1. Experimental design. At time 0, status epilepticus (SE) was induced in male Wistar rats via administration of lithium–pilocarpine. Two weeks after SE induction, the animals were treated with cerebrolysin (CBL; 538 mg/kg) or vehicle (0.9% NaCl) in a volume of 2.5 mL/kg for three weeks. At the end of treatment, the Barnes maze test (BMT) was conducted for one week, and one day thereafter, intracardial perfusion and brain dissection were performed. Additionally, the animals were monitored with video for five weeks to record the frequency and duration of spontaneous recurrent seizures.

consisted of one learning session per day over 5 consecutive days, and 72 h later, a final memory session was performed. Before starting the learning session, each rat was placed in the testing room for habituation for 30 min. The animal was then released in the center of the maze, and the time taken by the rat to find the escape cage was recorded as latency time. If the rat did not find the escape cage within 5 min, it was gently guided to it, and a latency time of 300 s was recorded. The number of errors or entries into incorrect holes was also quantified. Finally, the maze top and escape cage were cleaned with 70% ethanol and wiped dry. To determine the motor activity of the rats, ambulatory behavior (measured in cm/min) was evaluated on days 5 and 8 of the BMT.

2.5. Golgi–Cox staining method

One day after the BMTs were completed, the animals were intraperitoneally injected with sodium pentobarbital (60 mg/kg) to induce deep anesthesia. After, the animals were intracardially perfused with saline solution, the brains were manually removed and processed using the modified Golgi–Cox method, as described previously [33]. Hippocampal coronal sections (200 μm thick) were obtained using an electronic vibratome (OTS-4000, Electron Microscopy Sciences, Hatfield, PA, USA). The sections were placed on a clean gelatin-coated microscope slide, treated with ammonium hydroxide for 30 min, incubated for 30 min in a film fixer (Kodak, Rochester, NY, USA), rinsed with distilled water, and then mounted using a resinous medium [34].

2.6. Dendritic morphology and Sholl analysis

The Golgi-impregnated granular neurons of the DG of the dorsal hippocampus were localized according to the *Paxinos and Watson Atlas* [35] using the coronal brain plates 27 to 36 (Fig. 2). The granular cells were identified by the elliptical soma with a characteristic cone-shaped dendritic tree. The following criteria were used to select neurons for reconstruction: location in the region of interest in the hippocampus (Fig. 2) and in the middle 200 μm width of the section, complete impregnation of the neurons, and no morphological changes attributable to Golgi–Cox staining [36]. Neurons from each hemisphere were observed and delineated under a microscope using an integrated tablet (VE-B6PAD, VelaQuin, México). Ten neurons per rat were observed and identified at 380× magnification; the three-dimensional dendritic tree was reconstructed by drawing each neuron in a two-dimensional plane. Thereafter, the dendritic tree was subjected to Sholl analysis as follows. A transparent grid with concentric rings (equivalent to 10 μm between each ring) was placed over the dendritic drawing, and the number of ring intersections was used to calculate the total dendritic length. The total number of intersections was counted at each concentric ring away from the cell body to estimate dendritic arborization, and finally, the total number of dendritic branches was counted at each order away from the soma [37,38]. The amplitude of the dendritic tree was measured according to the angle formed by arborization, using the soma as the vertex. Dendritic spine density was quantified by drawing distal dendritic segments at high magnification (1560×) and counting the number of spines/50 μm [36]. Dendritic morphology analyses were performed by trained observers blinded to the experimental conditions.

2.7. Statistical analysis

The frequency of SRS is expressed as median (25th–75th percentile) and maximum values, whereas data regarding SRS duration and severity, BMT, and dendritic morphology analyses are expressed as mean ± standard error of the mean (SEM).

Comparisons of seizure incidences in epileptic and treated epileptic rats were performed using the Mann–Whitney *U* test and the Kruskal–Wallis test, followed by post hoc Dunn's tests. Data regarding SRS duration and severity, BMT, and dendritic morphology were analyzed using two-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls (S–N–K) post hoc multiple comparison test; $P < 0.05$ was considered to be statistically significant. The variances of the data from the motor activity and Sholl measures were not homogeneous; thus, they were transformed (square root) before being subjected to ANOVA.

3. Results

3.1. Effect of CBL on SRS

Seven days after SE induction, monitoring of SRS commenced, and in the following week, no seizures were detected in either of the two groups. Two weeks after SE induction (i.e., week 3), some rats from both epileptic groups exhibited SRS; however, statistical analysis did not reveal a significant difference between the groups, with median numbers of 1 (EPI) and 0 (EPI-CBL) SRS per week (Fig. 3). Over the next two weeks of treatment and on the behavioral test week, the EPI

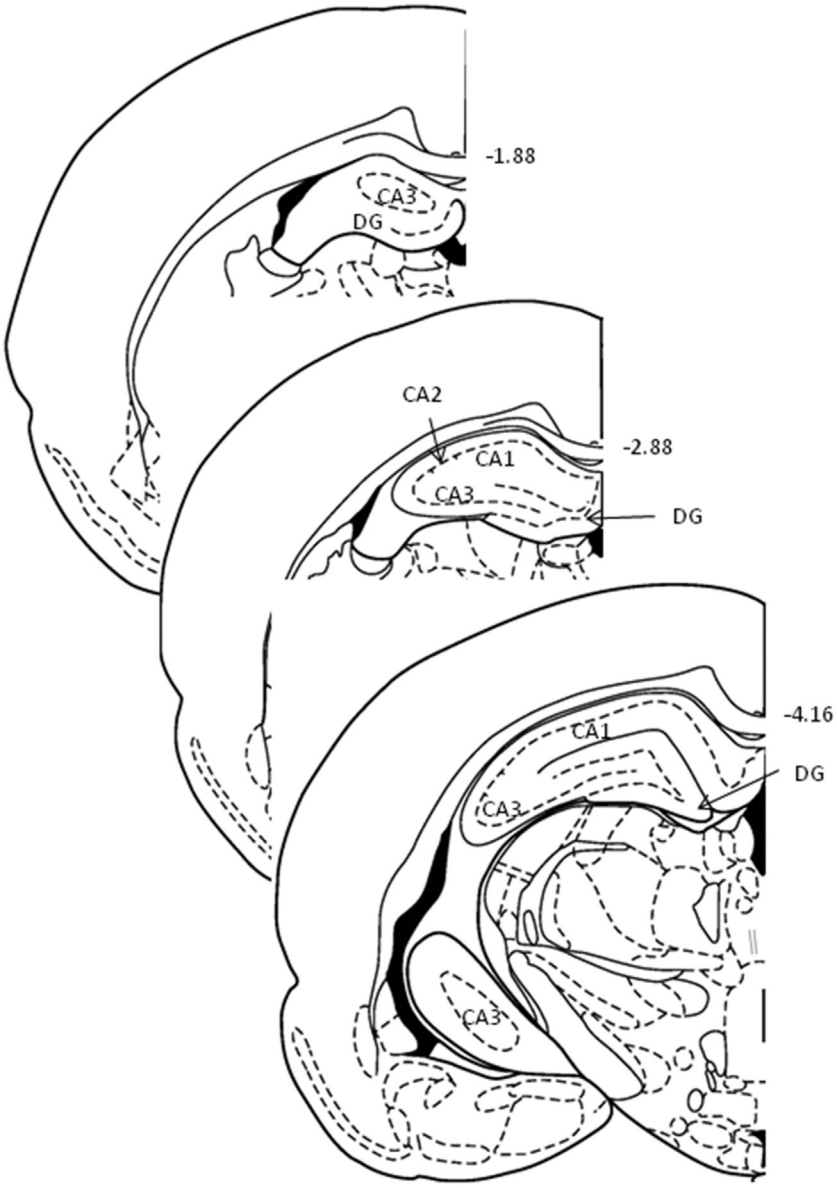

Fig. 2. Schematic drawing of coronal brain plates illustrating the hippocampal region of interest. Granular neurons from the dentate gyrus (DG) of the hippocampus were reconstructed using the Golgi–Cox procedure. Distances (mm) from bregma are indicated (reproduced using the *Paxinos and Watson Atlas* [35]).

and EPI-CBL groups exhibited similar SRS frequencies. In contrast, the Kruskal–Wallis test, followed by post hoc Dunn's test, revealed that the frequencies of SRS of EPI and EPI-CBL groups in weeks 3–6 demonstrated statistically significant differences with regard to their respective pretreatment monitoring (week 2, EPI: $H_{(4)} = 11.2$, $P = 0.024$; EPI-CBL: $H_{(4)} = 11.8$, $P = 0.019$).

With regard to latency to the first spontaneous seizure, ANOVA did not detect differences: EPI and EPI-CBL rats had a latency of approximately 18 days for presenting stage 4 or 5 (Table 1). Among the total of observed seizures, EPI-CBL rats exhibited an increase in stage 5 seizure compared with EPI animals (48.6 ± 1.2% versus [vs.] 40.7 ± 6.2%), this class of seizures was also of longer mean duration (72.0 ± 4.6 s vs. 60.3 ± 10.1 s); although in both cases without reaching statistical significance (Table 1). The videos of control rats during the five-week period did not reveal any SRS.

3.2. Spatial learning and memory assessments

No rats from any of the experimental groups exhibited any signs of motor impairment. Two-way ANOVA failed to detect significant differences caused by treatment or epileptic condition in ambulatory activity on days 5 and 8. However, on days 5 and 8 of the BMT, animals in the EPI group demonstrated a trend toward increased ambulatory activity compared with CONT and CONT-CBL rats (Table 2); instead, only on day 5, EPI-CBL rats exhibited a slight trend toward increased activity compared with controls. All groups began using search strategies to solve the task that were random; on days 5 and 8 of training, the controls and EPI-CBL rats replaced the random approach to one in which the holes were serially investigated and in minor proportion navigated more directly to the escape cage location. In contrast, EPI rats attempted to locate the goal in a random fashion; excessive thigmotaxis (without investigating holes) also was observed.

The average latency to locate the escape cage in each of the 6-test days is shown in Fig. 4A. Two-way ANOVA detected significant differences for the main effects of treatment ($F_{3,179} = 29.15$, $P < 0.001$) and sessions ($F_{5,179} = 5.76$, $P < 0.001$). In addition, there was a significant interaction between the treatment and session factors ($F_{15,179} = 2.48$, $P = 0.003$). Both the CONT and CONT-CBL groups learned the task, and the latency to locate the escape cage diminished across the training period. The post hoc S–N–K test revealed that the latency times on

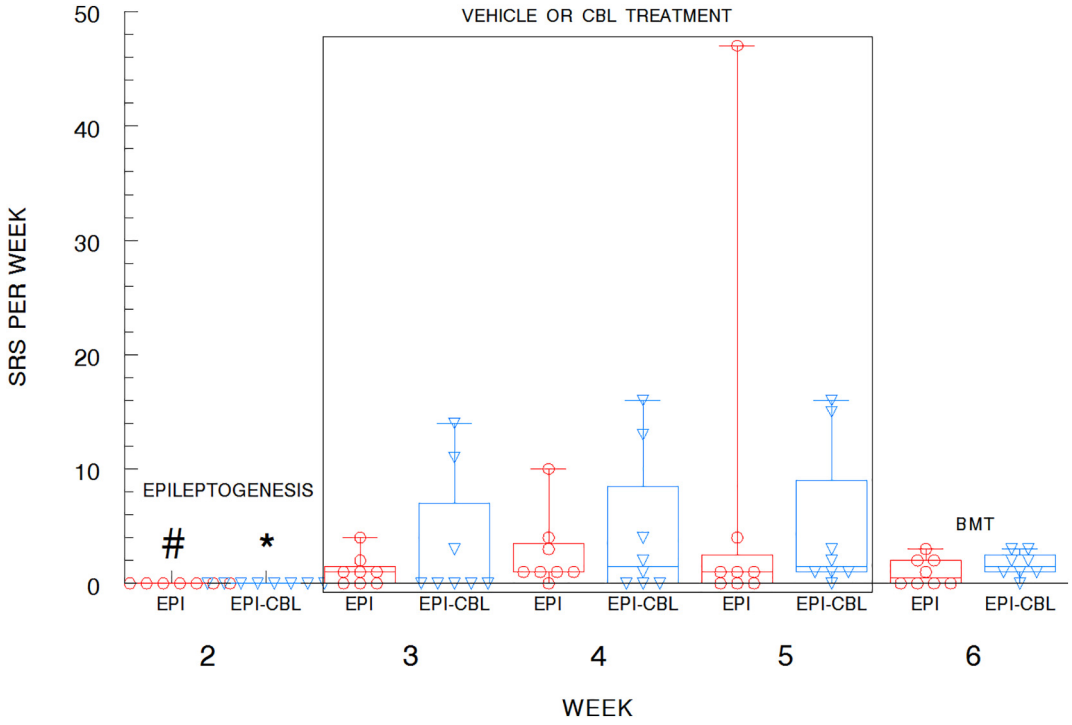

Fig. 3. Effect of cerebrolysin (CBL) treatment on the frequency of spontaneous recurrent seizures (SRS) in epileptic (EPI) rats. Data (horizontal bars represent medians, boxes represent 25th–75th percentiles, and vertical bars represent maximum values) are presented as the median number of SRS per week in EPI- (SSI [2.5 mL/kg]) and EPI-CBL-treated (538 mg/kg) rats. $P < 0.05$ versus weeks 3–6 in EPI (#) and EPI-CBL (*) groups, Kruskal–Wallis test followed by post hoc Dunn's tests ($n = 8$). BMT: Barnes maze test.

Table 1
Effect of cerebrolysin (CBL) treatment on spontaneous recurrent seizures (SRS) in epileptic (EPI) rats.

Group	Latency to the first spontaneous seizure (Days)	Stage 4 (%)	Duration (s)	Stage 5 (%)	Duration (s)
EPI	17.5 ± 1.0	59.3 ± 6.2	31.6 ± 8.5	40.7 ± 6.2	60.3 ± 10.1
EPI-CBL	18.4 ± 1.5	51.4 ± 1.2	41.4 ± 9.3	48.6 ± 1.2	72.0 ± 4.6

Data presented as mean ± standard error of the mean ($n = 8$). No statistical differences were found in the latency to the first SRS between EPI- (SSI [2.5 mL/kg]) and EPI-CBL-treated (538 mg/kg) rats. Similarly, differences in severity (percent of stage 4 and 5 on the Racine scale) and mean duration of SRS did not reach statistical significance between the groups.

training days 5 and 8 were significantly lower than that on day 1 of training. In contrast, the EPI rats did not exhibit spatial learning abilities, and their latency times were high throughout the training period. Interestingly, treatment with CBL improved acquisition performance in EPI-CBL rats, the latency time on training day 5 was significantly lower than that on day 1 of training, but did not reach the levels of CONT and CONT-CBL animals.

The S–N–K test also demonstrated that on training days 3, 4, 5, and 8, the latency times for the EPI and EPI-CBL groups were significantly different from the CONT and CONT-CBL groups; however, the latency in the EPI-CBL group was significantly lower than EPI group on training day 5. Learning in the BMT was also observed in the CONT, CONT-CBL, and EPI-CBL groups, as evidenced by a low number of errors or entries into incorrect holes during the training days (Fig. 4B). Two-way ANOVA revealed significant differences among treatments ($F_{3,179} = 23.39$, $P < 0.001$), but not for the session factor ($F_{5,179} = 1.11$, $P = 0.360$); however, the interaction between both factors was statistically significant ($F_{15,179} = 2.01$, $P = 0.018$). The S–N–K post hoc test revealed that in the EPI group, the number of errors increased across the sessions; however, the number of errors was lower in the other groups, with no significant differences among them (Fig. 4B).

Table 2
Locomotor activity in the Barnes maze test.

Day	CONT	EPI	CONT-CBL	EPI-CBL
5	306.6 ± 61.9	362.4 ± 74.7	287.0 ± 26.6	322.1 ± 69.5
8	283.6 ± 60.6	363.3 ± 76.0	296.0 ± 35.6	277 ± 46.5

Ambulatory activity (cm/min) on days 5 and 8 of the Barnes maze test. Data presented as mean ± standard error of the mean in control (CONT) and epileptic (EPI) saline-treated groups; and CONT-cerebrolysin (CBL) and EPI-CBL-treated rats ($n = 7–8$).

3.3. Dendritic morphology

Dendritic arborization and spine density of the neurons were measured as previously reported [36,37]. Golgi–Cox staining clearly filled the dendritic shafts and spines of granular cells in the DG of the dorsal hippocampus (Fig. 5). A total of 300 neurons from 30 rats were drawn, and the measurements from Sholl analysis included dendritic neuronal arborization as determined by the quantification of the number of intersections at each concentric ring away from the soma, the total dendritic length, and the dendritic length for each branching order. In granular neurons of the DG, two-way ANOVA revealed main effects of the radius of the shell ($F_{34,1049} = 152.49$, $P < 0.001$) and treatment ($F_{3,1049} = 13.16$, $P < 0.001$), but not for the interaction ($F_{102,1049} = 0,80$, $P = 0.929$), and pairwise comparisons revealed a reduction in dendritic arborization of the EPI rats compared with that of the CONT and CONT-CBL rats ($P < 0.05$), detecting differences from the soma

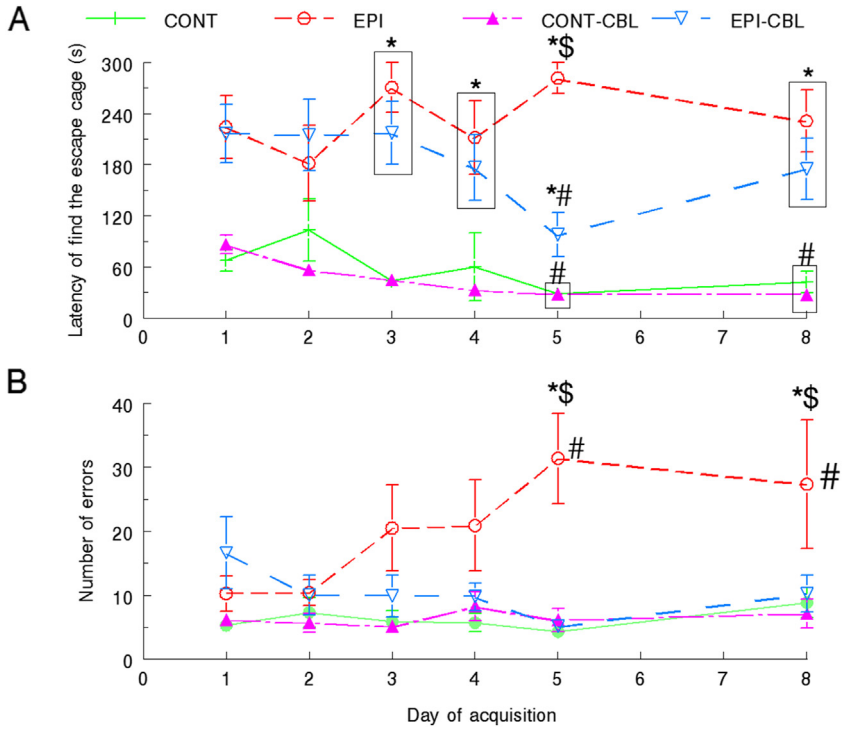

Fig. 4. Effects of cerebrolysin (CBL) treatment on latency to find the escape cage (A) and escape errors (B) in the Barnes maze test. Data presented as mean ± standard error of the mean in control (CONT) and epileptic (EPI) saline-treated groups as well as in the CONT-CBL and EPI-CBL-treated (538 mg/kg) rats. * $P < 0.05$ versus (vs.) CONT and CONT-CBL groups; $ $P < 0.05$ vs. EPI-CBL group; # $P < 0.05$ vs. its respective day 1 ($n = 7–8$).

from 170 to 210 μm. Interestingly, EPI-CBL rats increased dendritic arborization at the level of CONT rats (Fig. 6A), and CONT-CBL rats exhibited arborization similar to the CONT group.

An additional measure obtained from Sholl analysis was the length per branch order, with two-way ANOVA revealed main effects of treatment ($F_{3,209} = 2.86$, $P = 0.038$), branch order ($F_{6,209} = 96.54$, $P < 0.001$), and the interaction between these factors ($F_{18,209} = 1.86$, $P = 0.022$). Pairwise comparisons revealed that, at the third order, EPI rats showed reduced dendritic length compared with the CONT group ($P < 0.05$); however, CBL treatment in EPI-CBL rats did not alter this reduction (Fig. 6B).

At the fourth order, an increase in the length of the CONT-CBL and EPI-CBL groups was observed; however, this increase was only significant compared with the EPI group.

Another measure evaluated using Sholl analysis was total dendritic length (Table 3). Epilepsy caused a reduction in this variable; nevertheless, ANOVA did not detect significant differences caused by the treatment or epilepsy. Similar results were obtained for the angle of arborization (Table 3). Finally, dendritic spine density in the distal portions of granular cells was not affected by epilepsy or CBL treatment (Table 3).

4. Discussion

4.1. Cerebrolysin did not exert an anticonvulsant effect against secondarily generalized seizures

The results of this study did not demonstrate a protective effect of CBL against secondarily generalized seizures induced by pilocarpine. After SE in both epileptic groups, SRS (stages 4 or 5) appeared at approximately the same time (18 days). Moreover, epileptic animals treated with CBL exhibited seizures, similar in frequency, duration, and intensity, to those

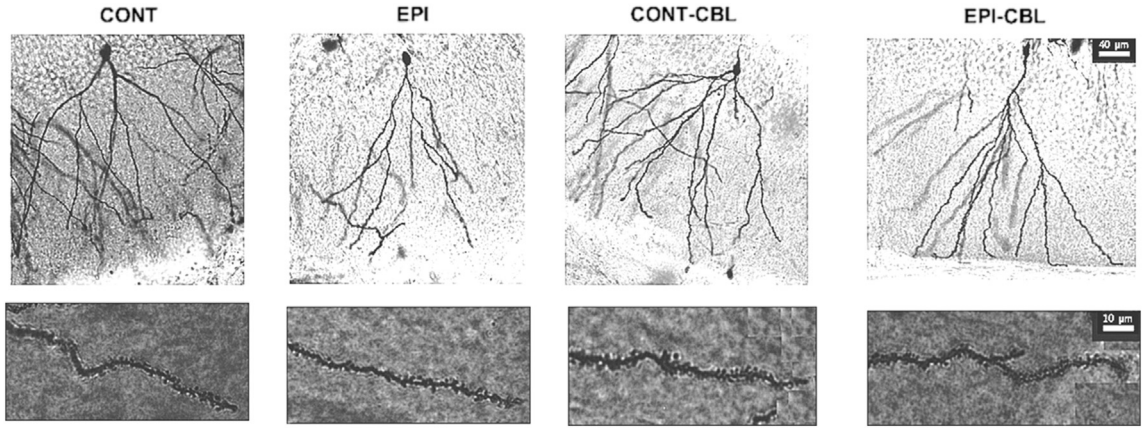

Fig. 5. Photomicrographs revealing Golgi–Cox impregnated dendritic tree and dendritic spine density of granular neurons of the hippocampal dentate gyrus in control (CONT), epileptic (EPI), control cerebrolysin-treated (CONT-CBL), and epileptic CBL-treated (EPI-CBL) rats.

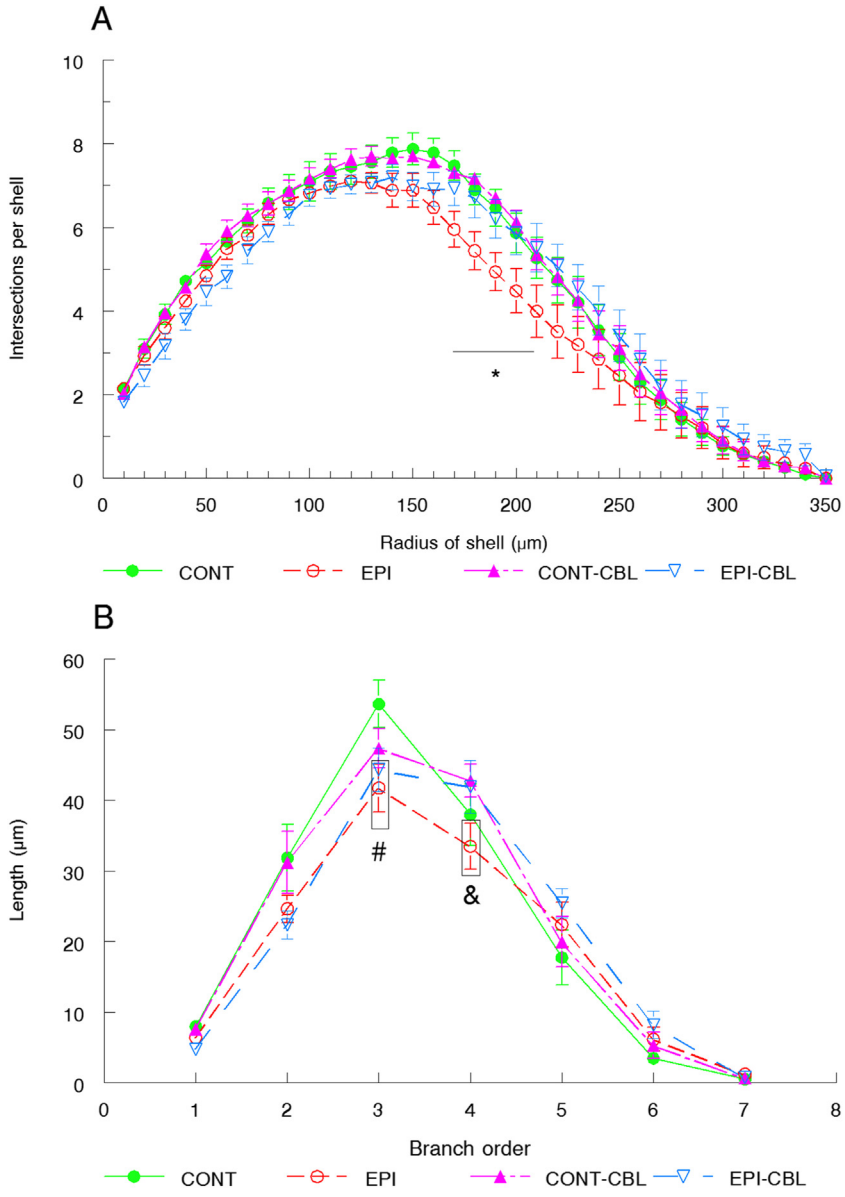

Fig. 6. Morphological Sholl analyses of dendritic arborization of granular neurons of the dorsal dentate gyrus in control (CONT), epileptic (EPI), and control cerebrolysin-treated (CONT-CBL) and epileptic CBL-treated (EPI-CBL) rats. A. The total number of intersections was evaluated at each concentric ring away from the cell body. * $P < 0.05$ versus (vs.) EPI-CBL and control groups. B. Branch order analysis of dendritic length. # $P < 0.05$ vs. CONT group; & $P < 0.05$ vs. CONT-CBL and EPI-CBL groups ($n = 7–8$).

of epileptic animals treated only with vehicle. This finding contradicts results reported by Gromova et al. [26], who used a primary generalized seizure model in which CBL was administered before SE. This discrepancy can be explained by the fact that CBL treatment in the present study started during the chronic period of TLE. However, it is important to note that, albeit in our study, CBL treatment did not alter the course and expression of epilepsy; there was a nonsignificant trend to augment frequency, duration, and intensity of SRS. The TLE animal model is more similar to epilepsy and is characterized by structural, biochemical, and behavioral changes that replicate the natural course of symptomatic focal epilepsy [4]. In fact, it has even been reported that CBL can contribute to the exacerbation or production of seizures, which are important side effects in patients with traumatic brain injury [27]. A possible explanation for this phenomenon is the well-known neurotrophic effects of CBL (reviewed in [17]), in which glutamatergic synapses in limbic structures can be induced to sustain or worsen epilepsy [39]. In addition, nonconvulsive seizures were not considered in the present study; thus, future studies are needed to reconcile the possible proconvulsive effect of CBL.

4.2. Epileptic cerebrolysin-treated rats improved cognitive performance in the Barnes maze

Cognitive impairments are common in patients with TLE [9], and the hippocampus, a structure involved in memory processes, is typically affected. In animal models of TLE, learning and memory impairments associated with changes in synaptic hippocampal plasticity have also been

Table 3
Dendritic morphology.

Variable	CONT	EPI	CONT-CBL	EPI-CBL
Arborization angle (°)	79.3 ± 4.4	71.0 ± 4.9	72.5 ± 5.5	67.5 ± 3.9
Total dendritic length (μm)	1532 ± 80.3	1362 ± 82.6	1557 ± 33.1	1480 ± 72.0
Spine density (spines/50 μm)	26.2 ± 0.7	27.4 ± 1.4	28.4 ± 1.4	28.2 ± 1.0

Data presented as mean ± standard error of the mean in control (CONT) and epileptic (EPI) saline-treated groups, as well as the CONT-cerebrolysin (CBL) and EPI-CBL-treated rats ($n = 7–8$).

reported [40]. In particular, several studies have related the DG with learning and memory processes [13,14,41–43]. Interestingly, the particular area of the DG evaluated here is a part of the dorsal hippocampus, a region that exhibits dendritic alterations with aging [36]. It is known that aging causes neuronal atrophy, which, consequently, leads to cognitive deficits. Our results are consistent with this fact: epileptic rats exhibited cognitive impairments in the BMT. Notably, treatment with CBL improved learning and memory processes in epileptic rats without reaching control levels. This improvement caused by CBL on days 5 and 8 of the BMT could, in part, be explained by the reduction in locomotor activity. On these days in the BMT, EPI rats exhibited an increase (statistically nonsignificant) in ambulatory activity and excessive thigmotaxis compared with controls; instead, only on day 5, EPI-CBL rats exhibited a slight trend toward increased activity compared with controls. However, EPI-CBL rats used different search strategies to solve the task compared with epileptic animals. In epileptic animals treated with CBL, the holes were serially investigated and, in minor proportion, were navigated more directly to the escape cage location. In contrast, epileptic rats attempted to locate the goal in a random fashion.

The improvement caused by CBL in learning and memory processes is consistent with investigations involving rats with bilateral lesions of the frontoparietal (sensorimotor) cortex, in which the administration of CBL attenuated acquisition deficits in the water maze task [44]. Cerebrolysin has neurotrophic effects similar to endogenous neurotrophic factors such as brain-derived neurotrophic factor, nerve growth factor, insulin-like growth factor, ciliary neurotrophic factor, and glial cell-derived neurotrophic factor [17,45]. In addition, CBL increased the expression of plasticity-related synaptic proteins, such as postsynaptic density protein 95, protein kinase C-gamma, p-CREB, and decreased the expression of apoptosis-related proteins in the hippocampus [46]. All of these factors have been shown to promote neuronal proliferation, survival, and synaptic plasticity, as well as inhibit apoptosis, which results in improved integrity of neuronal circuits. All of these effects of CBL could explain its beneficial effects on cognitive deficits in rats with TLE.

4.3. Cerebrolysin attenuated hippocampal granular neuron deterioration caused by epilepsy

Regarding the morphology results, it was found that epilepsy reduced dendritic arborization without altering dendritic spine density in granular cells of the DG. Dendritic degeneration is a common characteristic of neurons in epileptic tissues of both humans and animals [47,48]. The DG of the hippocampus is a structure that is closely associated with epilepsy because during epileptogenesis, it exhibits an important neuronal reorganization (mossy fiber sprouting in granular cells and the loss of hilar gamma-Aminobutyric acid (GABA) neurons) that results in the subsequent emergence of SRS in the animal model of pilocarpine-induced TLE [11,39]. To the best of our knowledge, there have been no studies using Golgi–Cox staining to evaluate the direct effects of repeated seizures on dendritic arborization in the granular cells of the DG. However, there is information regarding dendritic spine density. In the rat pilocarpine model, generalized spine loss has been observed in granular cells of the DG after acute SE; however, during the chronic phase, spine density recovers, which is consistent with our results [47,49,50]. Interestingly, it was also demonstrated that subchronic CBL treatment alleviated dendritic retraction caused by secondarily generalized seizures. Epileptic CBL-treated rats exhibited less alteration in dendritic arborization than saline-treated epileptic rats, although some dendritic retraction persisted, especially at the third branch order level. This partial remodeling may explain why the improvement in the cognitive performance of these CBL-treated animals did not reach control levels. This favorable effect of CBL has also been reported in aged mice: CBL administration restored dendritic length and dendritic spine density in the limbic regions [18,19].

Although the exact mechanism of CBL in neurons is currently unclear, CBL likely protects against dendritic degeneration by improving synaptic plasticity through its neurotrophic properties. It appears that the improvement in neuronal plasticity is preferentially directed to circuits related to memory and learning processes; however, the circuits involved in epileptic activity are not impacted. Recently, similar results were obtained in a lithium–pilocarpine model: early post-SE administration of the endocannabinoid agonist WIN-55,212-2 attenuated behavioral alterations and decreased neurodegeneration in the hippocampus but did not prevent the development of seizures [51]. Further investigations, therefore, are required.

5. Conclusions

Cerebrolysin did not exert an anticonvulsant effect against secondarily generalized seizures; however, CBL can be proposed for use as an add-on therapy in epilepsy management to prevent neuronal alterations, and to improve memory and learning processes.

Declaration of Competing Interest

We have no conflict of interest to declare.

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