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The Systemic Administration of Na^+/H^+ Exchanger-1 Inhibitor Impairs Hippocampal Learning and Memory

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ABSTRACT

Objective: Disruptions in pH regulation are known to cause impairments in cognitive processes such as learning and memory, leading to various neurological disorders. Na^+/H^+ exchangers (NHEs), embedded in the membranes of neurons, are crucial for regulating intracellular and extracellular pH. This project aims to investigate the effect of cariporide, a potent inhibitor of NHE1, on spatial learning performance.

Materials and Methods: A total of 20 male Wistar rats weighing between 240 g and 360 g were divided randomly into two groups: control (n=10) and cariporide (n=10). The cariporide group received a dosage of 10 mg/kg of cariporide added to the drinking water of the rats for three weeks. The Morris Water Maze (MWM) test was conducted on all groups. Distance moved (DM), escape latency (EL), swimming speed (SS), mean distance to the platform (MdtP), and time spent in the target quadrant (TSTQ) of the rats were recorded using the monitoring and recording system.

Results: The DM to find the platform and EL time did not show significant differences between the groups ($p>0.05$). The SS of the rats in the cariporide group significantly decreased compared to the control group on all days ($p<0.05$). The MdtP was statistically significantly increased in the cariporide group on the 2nd and 4th days.

Conclusion: The findings of this study indicate that systemic inhibition of NHE1 with cariporide affects hippocampal plasticity, leading to impairments in learning and memory. To elucidate its role in neurodegenerative diseases, further research should investigate cariporide's effect on hippocampal synaptic plasticity.

Keywords: Cariporide, hippocampus, learning, memory, Morris Water Maze.

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INTRODUCTION

The concentration of hydrogen ions (H^+) is one of the crucial parameters that must be maintained within specific limits for a cell to survive. The maintenance of a narrow range of hydrogen ion concentration is essential for various physiological processes, including biochemical reactions, optimal intracellular enzyme function, and the preservation of protein structure and functions. The pH balance between intracellular and extracellular environments is essential for the maintenance of physiological functions. Numerous mechanisms play a role in maintaining this balance, including physiological buffers, acid-base transporters, signal transduction pathways, and other proteins.[1]

Neuronal excitability and neurotransmission are potent metabolic processes that can cause drastic changes in intracellular pH.[2] These pH changes affect the membrane's electrical activity by regulating the permeability of various voltage-gated, proton-gated ion channels, and pH-sensitive neurotransmitters.[3] Previous studies have demonstrated that ischemia-induced changes in brain pH lead to neuronal cell death.[4,5] Additionally, Rathje et al.[5] demonstrated that stimulation of N-methyl-D-aspartate (NMDA) receptors leads to an increase in cellular Ca^{++} and H^+ concentrations. Although this combined effect can lead to excitotoxic cell death, it has been argued that Na^+/H^+ exchangers (NHEs) restore cellular pH to normal values within seconds. First identified by Sardet et al.[6] in 1989, NHEs are defined as secondary active membrane transporters and constitute an important group of transport proteins that protect cells against acidosis. NHEs are responsible for the uptake of Na^+ ions into the intracellular compartment through secondary active transport and the removal of H^+ ions from the cytoplasm at a ratio of 1:1 or 2:2[7] NHEs (1-9) have various isoforms; the most common isoform in the brain is NHE-1, which is mostly found in the hippocampus and cortical regions. NHE-1 is essential for cell differentiation and proliferation, controlling the fluid volume of cells, and maintaining pH levels within normal ranges in coordination with bicarbonate transporters.[8,9]

The hippocampus is a critical region of the brain, playing a central role in various functions, particularly in the formation of new memories and spatial navigation. The Morris Water Maze[10] (MWM) is a test designed to evaluate spatial learning associated with the hippocampus, based on repeated trials of learning the location of a platform placed in a water tank and subsequently recalling the learned location. In this study, we investigated the impact of NHEs on hippocampal functions. Dietrich et al.[11] demonstrated that synaptic acidification enhances gamma-aminobutyric acid-A (GABA-A) signaling. Although their study focused on cerebellar granule cells, in contrast to our study, they demonstrated that NHEs alter the amplitude and kinetics of GABA channel currents during synaptic transmission. Another study investigated the effects of NHE inhibition with cariporide on striatal dopaminergic transmission. Researchers demonstrated that the intra-striatal delivery of an NHE inhibitor resulted in an initial increase followed by a decrease in dopamine (DA) overflow, accompanied by a concurrent decline in striatal DA content.[12] The present study, in contrast to the mentioned neuronal paths, is related to the glutamatergic synapses in the hippocampal perforant path.

Cariporide (HOE 642) is a new, selective inhibitor of the Na^+/H^+ exchangers.[13] Research on cariporide is frequently concerned with reducing damage caused by ischemia-reperfusion after myocardial infarction.[14,15] However, studies demonstrating the effect of cariporide on neuromodulation are limited. Considering the information above, it is evident that intracellular and extracellular proton concentrations can change under certain pathological conditions or due to prolonged physiological activity. These changes may alter NHE activity, affecting kinase-phosphatase activities associated with plasticity in hippocampal neurons, thereby influencing hippocampal functions. However, the impact of inhibiting NHE1 on intracellular-extracellular pH changes or other functions of NHE1 related to hippocampal learning and memory has not yet been investigated. In this study, a potent NHE1 inhibitor, cariporide, was used to understand the function of NHE1.

MATERIALS AND METHODS

Experimental Animals

A total of 20 male Wistar rats weighing between 240 g and 360 g were used for all experiments. The animals were housed in the Experimental Research Application and Research Center under a 12-hour dark/light cycle, with the room temperature maintained between 19 °C and 22 °C. All protocols and procedures utilized were approved by the Animal Experiments Ethics Committee of Erciyes University (Approval No: 19/028, Date: 13.02.2019). Rats were group-housed with standard rodent chow and water available ad libitum. The animals were randomly divided into two groups, with 10 rats in each: the control group (n=10) and the cariporide group (n=10). To form the cariporide group, 10 mg/kg of cariporide (Sigma-Aldrich) was added to the drinking water of the animals for three weeks.

Morris Water Maze

The Morris Water Maze test was performed using a stainless steel cylindrical water tank with a diameter of 180 cm and a height of 75 cm. The tank was filled with water to a depth of 55 cm, and its temperature was maintained at 23±2 °C. To make it more challenging for rats to identify platforms, colorful ink was added to the water. An analog camera was installed on the ceiling directly above the center of the tank to record the animals' movements within the tank during swimming. The recordings were automatically analyzed using the Noldus Ethovision-XT18 camera tracking system (Noldus, Leesburg, VA).

The five-day Morris Water Maze test was divided into two phases: a learning phase lasting four days with four trials per day, and a memory consolidation test consisting of just one trial (probe). During the four days of learning trials, rats were placed into the water from four different quadrants each day, at 20-minute intervals. After being released into the tank, the rats were expected to find the hidden platform, positioned 2 cm below the water surface in the target quadrant. If a rat failed to find the platform within one minute, it was assisted to locate it and allowed to stay there for 20 seconds. On the 5th day, the hidden platform was removed from the water tank, and the time spent in the quadrant where the platform had

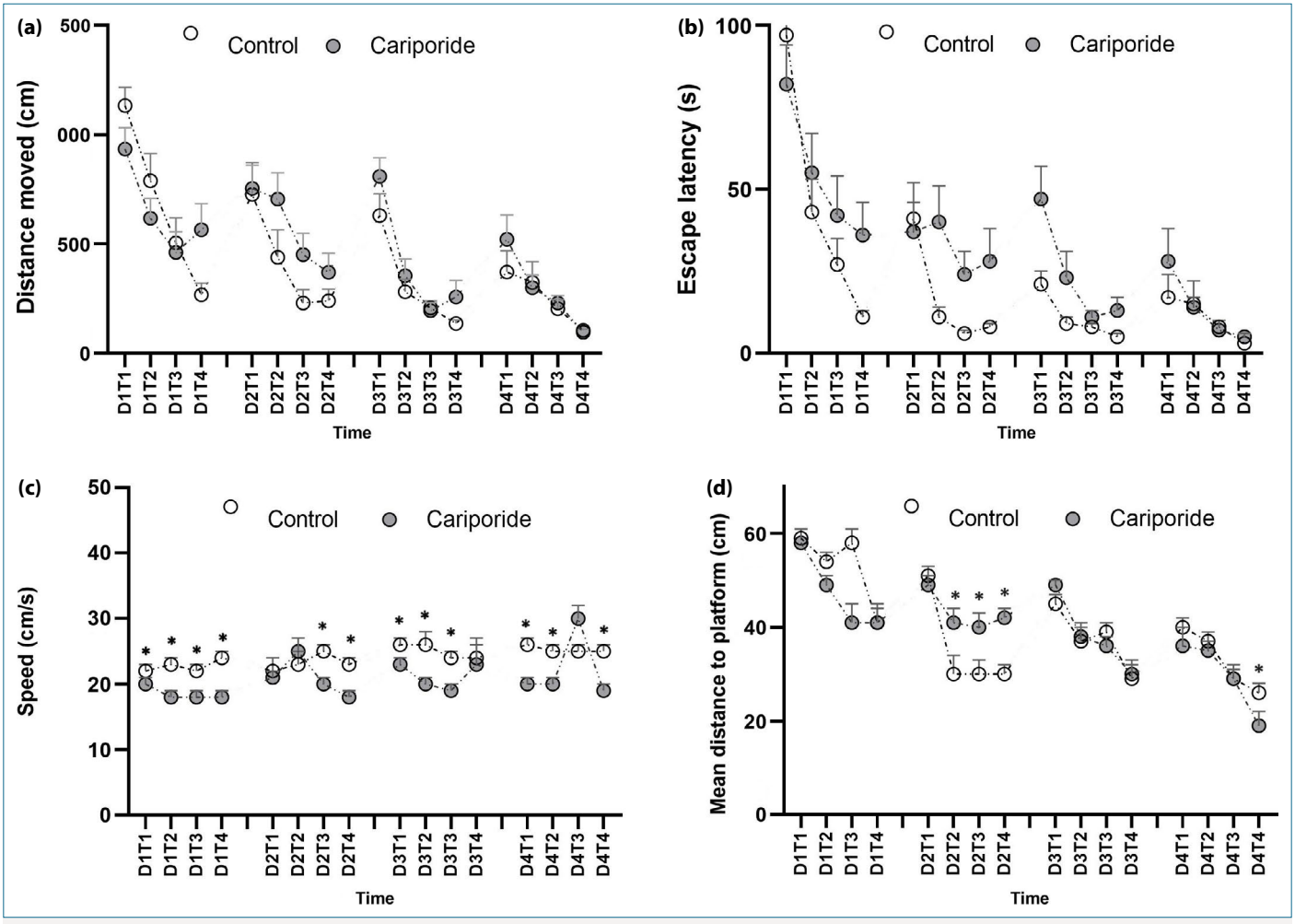

Figure 1. Effect of cariporide on (a) distance moved, (b) escape latency, (c) swimming speed, and (d) mean distance to the platform during four days of training in the Morris water maze.

Values represent the mean±SEM from 10 adult male rats in each group. An asterisk (*) indicates a difference from the control group.

previously been located was calculated as a percentage. The swimming speed (SS), escape latency (EL), distance moved (DM), mean distance to the platform (MdtP), and time spent in the target quadrant (TSTQ) were recorded using the NOLDUS tracking and recording system.

Data Analysis

The statistical analysis of the Morris Water Maze Test results was performed using the Statistical Package for the Social Sciences (SPSS) Version 16 software on a Windows 8 computer. The sample size for each group was determined to achieve 80% test power, using the G*Power program before the study commenced. Two-way repeated measures (RM) Analysis of Variance (ANOVA) was also applied to analyze the learning in the MWM training phase for each session, with treatment type and testing day as factors. Tukey's multiple comparisons post-hoc test was used to determine whether the animals' performance significantly differed across the training days. Further, to analyze the time spent in the target quadrant during the probe trial, an independent samples t-test against a hypothetical value was used. The normality of the data was assessed using the Shapiro-Wilk test. The results of these tests demonstrated that the data exhibited homogeneous variance. A p-value of <0.05 was considered indicative of statistical significance.

RESULTS

The total distance moved showed statistically significant differences for Day ($F=27.534$, $p<0.001$) and Trial ($F=59.467$, $p<0.001$). However, the Day*Trial interaction ($F=1.518$, $p=0.141$) was not statistically significant. As seen in Figure 1a, the distance moved to find the platform significantly decreased from Day 1 to Day 4, indicating a statistically significant learning effect.

Table 1. Comparison of results in the Morris Water Maze test between groups

	D1T1	D1T2	D1T3	D1T4	D2T1	D2T2	D2T3	D2T4	D3T1	D3T2	D3T3	D3T4	D4T1	D4T2	D4T3	D4T4
Control																
DM	1133±84	789±125	506±113	268±53	727±145	439±125	231±61	240±54	630±99	282±57	208±32	137±16	372±97	324±95	204±41	106±4
EL	97±10	43±10	27±8	11±2	41±11	11±3	6±1	8±1	21±4	9±2	8±1	5±1	17±7	15±7	8±2	3±1
SS	22±1	23±1	22±1	24±1	22±2	23±2	25±1	23±1	26±1	26±2	24±1	24±2	26±1	25±1	25±1	25±1
MdtP	59±2	54±2	58±3	41±4	51±2	30±4	30±3	30±2	45±2	37±3	39±2	29±3	40±2	37±2	29±3	26±2
Cariporide																
DM	934±98	618±90	462±93	566±11	754±106	706±119	452±97	371±87	809±85	355±77	195±37	258±75	522±111	300±58	231±33	97±16
EL	82±12	55±12	42±12	36±10	37±9	40±11	24±7	28±10	47±10	23±8	11±2	13±4	28±10	14±3	7±1	5±1
SS	20*±1	18*±1	18*±1	18*±1	21±2	25±2	20*±1	18*±1	23*±1	20*±1	19*±1	23±4	20*±1	20*±1	30±2	19*±1
MdtP	58±1	49±2	41±4	41±3	49±2	41*±3	40*±3	42*±2	49±1	38±3	36±2	30±3	36±4	35±2	29±2	19*±3

Values represent the mean±SEM from 10 adult male rats in each group. An asterisk (*) indicates a difference from the control group. DM: Distance moved; EL: Escape latency; SS: Swimming speed; MdtP: Mean distance to the platform.

The t-test analysis indicated that there were no statistically significant differences between the groups.

The escape latency for groups showed statistically significant differences for Day (F=24.767, p<0.001) and Trial (F=31.933, p<0.001) (Fig. 1b). However, the Day*Trial interaction (F=2.207, p=0.063) was not statistically significant. The t-test analysis indicated that there were no statistically significant differences between the groups.

As seen in Figure 1c, swimming speed did not show statistical significance for Day (F=3.780, p=0.013) and Trial (F=1.635, p=0.187). However, the Day*Trial interaction (F=4.593, p<0.001) was statistically significant. Group comparisons for the Day*Group (F=0.256, p=0.857) and Trial*Group (F=1.327, p=0.270) interactions did not show statistical significance. The Day*Trial*Group variable interaction was found to be statistically significant (F=44.49, p<0.001). The cariporide group swam more slowly than the control group on the 1st day's 1st, 2nd, 3rd, and 4th trials (p<0.05), the 2nd day's 3rd and 4th trials (p<0.05), the 3rd day's 1st, 2nd, and 3rd trials (p<0.05), and the 4th day's 1st, 2nd, and 4th trials (p<0.05).

The mean distance to the platform showed statistical significance for Day (F=39.337, p<0.001) and Trial (F=62.724, p<0.001) variables, and the interaction of Day*Trial (F=2.511, p=0.009) was also found to be statistically significant (Fig. 1d). The interaction between the Group and Day factors was found to be statistically significant, while the pairwise interaction with the Trial variable was not statistically significant (Day*Group: F=4.377, p=0.006; Trial*Group: F=0.257, p=0.940). In group comparisons, a statistically significant difference was observed between the cariporide group and the control group on the 2nd day's 2nd, 3rd (p<0.05), and 4th trials, as well as on the 4th day's 4th trial (p<0.05). These results indicate that learning was impaired in the group treated with cariporide. In the probe experiment on day 5, rats treated with cariporide spent less time in the target quadrant, but this decrease was not statistically significant (F= 8.831, p=0.176) (Fig. 2). All data for the groups are presented in Table 1.

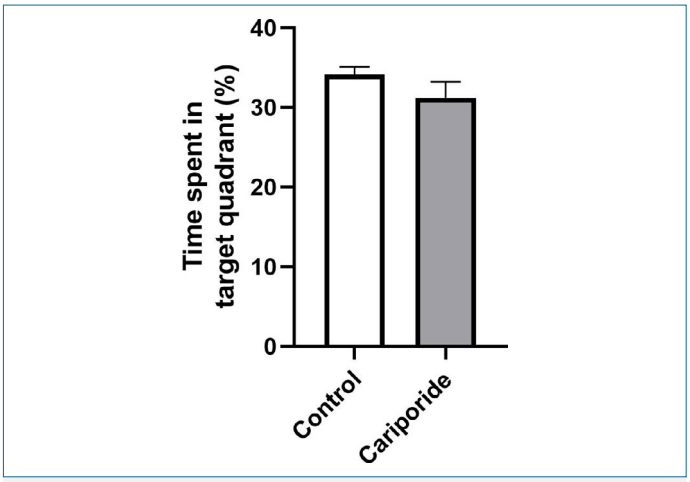

Figure 2. Duration groups spent in the target quadrant on the 5th day of the Morris Water Maze test.

Values represent the mean±SEM from 10 adult male rats in each group.

DISCUSSION

The concentration of H^+ ions is regulated by different mechanisms within the organisms to maintain the viability and physiological functions of cells, keeping the intracellular pH within narrow limits. Regulation of pH involves a variety of buffering systems and ion transport mechanisms, including those involving the lungs, kidneys, sodium-proton exchangers, chloride-bicarbonate exchangers, and sodium-bicarbonate

transporters.[16] The complexity of this regulation makes it difficult to understand the significance of each mechanism fully. The main finding of this study is that systemic inhibition of NHEs leads to impairments in hippocampus-dependent learning and memory functions. However, due to the systemic nature of cariporide treatment, it is difficult to discern whether this effect is direct or indirect.

Chen et al.[17] demonstrated that NHE5-deficient mice exhibit significantly enhanced cognitive performance in the MWM test, findings that are opposite to ours. However, the two investigations focus on different NHE isoforms and experimental animal species. NHE1[18] and NHE5[19] are highly expressed in the brain. Researchers have emphasized that, unlike the widespread distribution of NHE1, NHE5 is predominantly expressed in the synaptic compartment, suggesting its potential significance in synaptic transmission.[20] NHE1-5 are localized to the plasma membrane, whereas NHE6-9 are found at intracellular organelles. Mutations in NHE1 are associated with epilepsy, ataxia, and growth retardation.[21] On the other side, studies have shown that potassium-sparing diuretics, such as amiloride, improve cognitive functions in cases of doxorubicin-induced cognitive impairment.[22] Amiloride, a potassium-sparing diuretic, is thought to exert potential neuroprotective effects through its effect on specific ion transporters such as NHE, acid-sensing ion channels, and Na^+/Ca^{++} exchangers.

NMDA receptors are pivotal to the widely accepted concept explaining the molecular basis of learning and memory.[23] Activation of NMDA receptors leads to an increase in intracellular Ca^{++} and Na^+ ions, resulting in cellular excitation. In this study, the inhibition of NHE1 may have led to reduced extracellular H^+ ion efflux in hippocampal granule cells, potentially causing extracellular alkalosis. This alkaline shift in the extracellular fluid, or the decrease in proton density, may have resulted in the suppression of NMDA receptor activity. The altered pH concentration and ensuing acidosis may have led to disruptions in learning and memory functions by modifying the activities of signaling systems and enzymes within the cell. Under physiological conditions, an increase in intracellular calcium initiates a process that begins with the activation of calmodulin-dependent protein kinase II (CaMKII). CaMKII plays a significant role in the long-term potentiation phenomenon associated with learning and memory.[24] Many proteins involved in these processes, such as voltage-gated calcium channels, NMDA/alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, and GABA receptors, are activated in a pH-dependent manner. Changes in the activity of these channels can alter the activity of intracellular kinases.[25] It has been reported that proteins including Protein Kinase B (AKT), p38 mitogen-activated protein kinases (p38), Extracellular signal-regulated kinases (ERK), Ribosomal S6 Kinase (Rsk), and Rho-associated, coiled-coil containing protein kinase (Rock) are involved in the phosphorylation of NHE1. In another study, it was reported that under conditions of excessive glutamate release, acidosis occurs in the cell due to the activation of the Protein kinase C beta/Extracellular signal-regulated kinases 1-2/p90RSK pathway.[26] The mentioned studies emphasize the role of NHEs and pH regulation in learning and memory.

CONCLUSION

The findings of this study have demonstrated that the inhibition of NHE1 affects behavior tests related to hippocampal plasticity. Unlike the systemic administration of cariporide in our study, delivering it through infusion to hippocampal tissue may allow for more specific results. The identification of kinases involved in regulating NHE1 activity and their relationship with learning will clarify the role of NHEs in learning and memory.

The limitations of this study stem from the systemic administration of cariporide, which makes it difficult to determine its direct or indirect effects on the hippocampus. Furthermore, the absence of molecular studies precludes the identification of relevant hippocampal pathways in this study.

Ethics Committee Approval: The Erciyes University Animal Experiments Ethics Committee granted approval for this study (date: 13.02.2019, number: 19/028).

Author Contributions: Concept – ND, FC; Design – ND, FC; Supervision – ND, OY; Resource – OY; Materials – ND, OY; Data Collection and/or Processing – EB, FC; Analysis and/or Interpretation – EB, CS; Literature Search – EB, CS; Writing – EB; Critical Reviews – CS, ND.

Conflict of Interest: The authors have no conflict of interest to declare.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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