The effect of chronic stress and its preconditioning on spatial memory as well as hippocampal LRP1 and RAGE expression in a streptozotocin-induced rat model of Alzheimer's disease

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Abstract

According to available evidence, prolonged or chronic exposure to stress is detrimental to various brain structures, including the hippocampus. The current study examined the expression of two critical blood-brain barrier receptors required for amyloid-beta clearance to understand better the mechanism by which chronic stress impairs learning and memory in patients with Alzheimer's disease (AD). Rats were bilaterally injected with streptozotocin (STZ) (3mg/kg) using the intracerebroventricular (ICV) technique to induce the Alzheimer's model. Additionally, they were subjected to foot shock (1mA, 1Hz) for 10s every 60s (1h/day) for ten consecutive days prior to and following STZ injection. The Morris Water Maze (MWM) test was used to assess spatial learning and memory. Real-time PCR was used to determine Low-density lipoprotein receptor-related protein-1 (LRP1) and receptor for advanced glycation end-products (RAGE) mRNA levels in the hippocampus. Moreover, the animals' body weights were determined as physiological parameters in all groups. The results indicated that 10-day chronic electric foot shock stress reduced body weight, impaired spatial learning and memory, decreased hippocampal LRP1 mRNA expression, and increased hippocampal RAGE mRNA expression in a rat AD model. It can be concluded that chronic stress in conjunction with AD alters the expression of LRP1 and RAGE in the hippocampus. The findings pave the way for scientists to develop novel treatment strategies for AD.

Introduction

Globally, the prevalence of age-related diseases has risen to become a significant public health concern. These diseases are well-known for causing progressive and permanent neuron loss, which results in dementia. Alzheimer's disease (AD) is one of these diseases associated with aging (Mahdi et al., 2019; Yiannopoulou & Papageorgiou, 2020). The disease is a progressive neurodegenerative illness characterized by multiple cognitive impairments and memory loss caused by intracellular tau aggregates and extracellular amyloid-beta ($A\beta$) accumulation (DeTure & Dickson, 2019; Masters et al., 2015). The occurrence and prevalence of AD and other dementias have increased significantly over the last decade (Laurent, Ejtehadi, Rezaei, Kehoe, & Mahmoudi, 2012). Thus, discovering cures or successful long-term treatment for AD is an urgent concern for healthcare.

Multiple approaches, including chemicals, have been used to induce AD-like symptoms in rats to evaluate various therapeutic agents for a range of cognitive dysfunctions. STZ is one of the chemicals being studied to understand better the disease's etiology (Mahdi et al., 2019; More, Kumar, Cho, Yun, & Choi, 2016). Recently, experts proposed that AD is a type of type 3 diabetes of the brain. STZ-induced experimental brain diabetes shares numerous similarities with AD (De la Monte & Wands, 2008; Lester-Coll et al., 2006). ICV-STZ is an important animal model of chronic brain dysfunction, characterized by progressive deficits in learning, memory, and cognition, an increase in $A\beta$ -42, and a persistent and continuous cerebral energy deficit (Chen et al., 2013; Lester-Coll et al., 2006; Mahdi et al., 2019). Multiple doses of ICV-STZ are used to induce memory impairment in rats. Memory impairment is caused by injecting STZ (3 mg/kg) once daily on the first day and twice daily on the first and third days following STZ injection in the second week (Kamat, 2015; Mehla, Pahuja, & Gupta, 2013).

According to previous research, premature progressive AD may be caused by factors other than genetics and senescence (Machado et al., 2014). As per recent publications, environmental parameters such as chronic stress may contribute to the acceleration of AD pathogenicity. It has been reported that electric foot shock is used in animal models of depression, anxiety, and post-traumatic stress disorder (PTSD) as a validated model. Additionally, it is the most frequently used type of trauma in preclinical studies of PTSD (Bali & Jaggi, 2015a, 2015b). Increased exposure to environmental or psychological stressors is associated with the onset of AD, with these stressors playing a critical role in the disease's progression (Cuadrado-Tejedor et al., 2012; Srivareerat, Tran, Alzoubi, & Alkadhi, 2009). Numerous studies have demonstrated that chronic stress can impair memory in a rat AD model (Machado et al., 2014; Srivareerat et al., 2009). However, a complete understanding of the precise mechanism of action has not been attained.

The blood-brain barrier (BBB) is a diffusion barrier necessary for the proper function of the central nervous system (CNS). Along with the BBB, several receptors and transporters exist that enable molecules to pass through substrate-specific transport systems critical for AD pathogenesis (Govindpani et al., 2019; Weiss, Miller, Cazaubon, & Couraud, 2009). For example, Low-density lipoprotein receptor-related protein-1 (LRP1) expression is moderate in the normal BBB, whereas receptor for advanced glycation end-products (RAGE) expression is minimal (Deane, 2012; Sagare, Deane, & Zlokovic, 2012; Storck et al., 2016). According to a previous study, in STZ-induced Alzheimer rats, LRP1 was decreased, and RAGE was increased at the BBB (L. Wang, Liu, Fan, Liu, & Yu, 2017). Furthermore, it has been demonstrated that chronic or overwhelming stress has a detrimental effect on the brain, including the BBB (Kempuraj et al., 2019; Sántha et al., 2016; Urayama & Banks, 2006). While research on the acute and chronic effects of stress on BBB dysfunction has been conducted, specific molecular and ultrastructural alterations have received insufficient attention. As a result, these changes are poorly understood.

By examining BBB-mediated transportation, a better understanding of the mechanisms underlying neurological disorders such as stress and AD can be gained, allowing for the development of disease-specific and efficacious therapeutic alternatives. The current study examined the effect of AD and chronic stress on hippocampal LRP1 and RAGE expression through two distinct study designs.

Methods

Study design

Male Sprague-Dawley rats weighing 200–250 g were obtained from the Laboratory Animal Center of Shiraz University of Medical Sciences (SUMS). Then, the animals were maintained under the standard condition of a 12 to 12 h light-dark cycle at 25 ± 2 °C, and water and food were provided ad libitum. The experimental protocols were approved by the ethics committee of SUMS, and the animal care was according to the NIH Guide for the care and use of laboratory animals. The design of the study is represented in figure 1. In the present study, rats were randomly assigned to one of two stress groups prior to and following streptozotocin (STZ) intracerebroventricular (ICV) injection. Then, each main group

was subdivided into four subgroups (7 rats per group). Stress group before ICV injection of STZ include sham (rats received ICV normal saline), STZ (rats received ICV-STZ), Stress (For ten days), and Stress+STZ (animals were exposed to stress firstly, then received STZ). Furthermore, the stress group after ICV injection of STZ included sham (rats received ICV normal saline), STZ (rats received ICV-STZ), Stress (For ten days), and STZ+Stress (animals received STZ firstly; then they were exposed to stress). Their body weights were measured on the 1st and last day of the experiment, and Body Weight differences (BWD= final body weight–initial body weight) were reported (Radahmadi, Alaei, Sharifi, & Hosseini, 2013). Additionally, the Morris Water Maze (MWM) was used to evaluate spatial learning and memory. At the end of each experiment, the animals were sacrificed, and hippocampal LRP1 and RAGE expression were assessed by real-time PCR.

Surgical procedure

Rats were anesthetized on the operation date with an intraperitoneal injection of a ketamine (100 mg/kg) and xylazine (10 mg/kg) mixture. After mounting the animals into a stereotaxic frame, bilateral implantation of a stainless-steel guide cannula (22 gauge) was done into the lateral ventricle (AP-0.8, ML±1.5, DV-3.5) based on the paxinos brain atlas. Stainless screws and acrylic cement anchor these cannulas to the skull. In line with the literature, the rats receiving STZ (Sigma-Aldrich, Catalog No S0130 SIGMA) were subjected to two doses of 3mg/kg on the first and third days. The sham group underwent similar surgical procedures, but their injections were administered with an equivalent amount of saline instead of STZ (Negintaji, Zarifkar, Ghasemi, & Moosavi, 2015).

Stress protocol

A communication box was employed to deliver electrical foot shock stress. Transparent plastic sheets split this instrument into nine divisions. The rats were subjected to physical stress through an electrical foot-shock (1mA, 1Hz) at 10-second intervals every 60 seconds (1 h/day) for ten consecutive days through a stainless steel grid. In contrast, control animals were maintained in peace in their cages during the experiment. The control animals were then placed into the experimental room and handled the same way as the stress group, except receiving foot shock (Hormozi, Zarifkar, Tatar, Barazesh, & Rostami, 2018).

Evaluation of spatial learning and memory

The MVM test was used to assess spatial learning and memory. The apparatus consisted of a black round pool with a diameter of 140 cm and a height of 70 cm, filled to a depth of 25 cm with 20°C water. The maze was divided into four equivalent quarters, with release points projected as N, E, S, and W at each quarter. A concealed round platform (11 cm in diameter) was located in the center of the southwestern quarter and submerged in 1.5 cm underneath the water surface. Additional maze visual signals were placed at different places around the maze (i.e., a computer, a door, a window, bookshelves, and posters). A charged coupled device (CCD) camera was fixed overhead the center of the maze in such a way to record animal activities and to send them to the computer. A computerized system (Noldus

EthoVision, v13, Noldus Company, The Netherlands) was used to record the animal's swimming route automatically.

Procedure

The procedure consisted of training sessions for four days. An unobservable platform was submerged almost 1.5 cm beneath the water surface during the primary three successive days. A block session comprised four trials with four different beginning locations. During every trial, the animal was allowed for 90 s to discover the invisible platform. The rats were allowed to stay in the mounted platform for 20 s until the subsequent trial. The invisible platform was taken out, and the retention testing (probe trial) was implemented on the 4th day. Following the probe trial, an observable platform was positioned in another location to examine the rats' motivation, visual ability, and sensory-motor coordination (Zarifkar, Zarifkar, Nami, Rafati, & Aligholi, 2018). During days 1–3 of training, the swimming speed, escape latency to the hidden platform, frequency of entry into the target zone, and time spent in the target quadrant during the spatial probe test were measured.

Tissue collection and Real-time PCR analysis

After completion of the behavioral assessment, the animals were sacrificed. The hippocampus was quickly isolated on ice, was transferred to liquid nitrogen, and then stored in -72°C until the molecular analysis. According to the manufacturer's protocol, the total RNA of hippocampus tissue (100 mg in weight) was extracted using RNA Sol reagent (Alpha Bio®, Cat. No: RSL 0050). RNA Sol isolation reagent is a mixture of Guanidium and phenol, which effectively dissolves DNA, RNA, and protein on homogenization or lysis of tissue samples. Then, cDNA was synthesized using a cDNA synthesis kit (EURx, Cat. No: E0801-01) according to the manufacturer's instructions. An average of 500 ng/μl of the total RNA was used to synthesize copy DNA (cDNA) using Oligo (dT) 20 and random hexamer primers. The cDNA product was used in the RT-qPCR after the synthesis. All real-time PCR reactions were performed using SYBR Green real-time PCR kit (EURx, Cat. No: E0402-01). The real-time PCR conditions were as follows: initial activation at 95°C for 1 minute, 40 thermal cycles of denaturation at 95°C for 10 seconds, primer annealing at 60°C for 20 seconds, and DNA polymerase extension at 72°C for 30 seconds. Then, the melting curve analysis was 95°C for 15 seconds, and 60°C for 1 minute until 95°C for 15 seconds. The PCR reaction included 5 μl master mix PCR, one μl forward and reverse Primers (Stoke 1 pm), 1.9 μl water 0.1 μl Rox dye and 1 μl sample cDNA to a final volume of 10 μl. All qPCR reactions were performed in a StepOnePlus Real-Time PCR apparatus (Applied Biosystems), and gene expression levels were analyzed via the $\Delta\Delta C_t$ method. Gene expression levels of the housekeeping gene, Gapdh, were used in each reaction to normalize the values determined. The specific primer sequences for RAGE were forward 5-CTGGCACTTGGATGGGAAAC-3 and reverse 5-CTGTCCCTGTAGTGCGTATGA-3; for LRP1 they were forward 5-CACTATGGATGCCCTAAACT-3 and reverse 5-CTGGGCTTTACTCTGTGGAC-3. For standardization, Gapdh was used as a reference gene, and the sequence of its primers is as follows: forward 5-GAGCAAGAGAGAGGCCCTCA-3 and reverse 5-TTATGGGGTCTGGGATGGAA-3. Primer sequences were purchased from Pishgam co. (Iran) (Khodadadi et al., 2018).

Statistical analysis

The results were indicated as the mean $\pm$ SEM. Analyses were done by using SPSS software version 20. Data were analyzed by repeated measure and one-way analysis of variance (ANOVA) followed by a post hoc test for multiple comparisons. In all the assessments, $P < 0.05$ was regarded as statistically significant.

Results

Result of Stress group before ICV injection of STZ

Body weight difference

Figure 2 shows the BWD in the stress group before ICV injection of STZ during the study. There was no significant difference in BWD between the sham group and the stress group. On the other hand, this index dramatically decreased in the STZ and Stress+STZ groups compared to the sham and stress groups ($P < 0.05$).

Spatial learning and memory

The performance of the stress group before ICV injection of STZ in the MWM test is indicated in Figure 3. Figure 3A depicts the animals' learning abilities after three days of training. The groups had a significant difference in escape latency ($F(3, 24)=31.12$, $P < 0.01$), according to the repeated measure analysis. The mean escape latency in STZ groups (with or without stress) was substantially more prolonged than in sham and stress groups, according to a Post-hoc Tukey's test. Figure 3B depicts the frequency with which animals enter the platform area and its vicinity. A significant difference between groups was observed using one-way ANOVA ($F(3, 24)=12.04$, $P < 0.01$). The frequency of access into the platform area and its vicinity in the Stress+STZ and STZ groups was significantly different from the sham group, according to a Post-hoc Tukey's test. The time spent in the platform area and its vicinity are depicted in Figure 3C. A significant difference between groups was observed using one-way ANOVA ($F(3, 24)=37.19$, $P < 0.01$). The time spent at the platform area and its vicinity in the Stress and STZ groups (with or without stress) differed substantially from the sham and stress groups, according to a Post-hoc Tukey's test. The mean swimming velocity during training days is depicted in Figure 3D. The one-way ANOVA of swimming speed revealed no significant differences between groups ($F(3, 24)=0.4$, $P=0.76$), indicating that swimming speed did not affect the animal's performance. The escape delay in reaching the visible platform is depicted in Figure 3E. The results of a one-way ANOVA test on the escape latency to the visible platform revealed no significant differences between groups ($F(3, 24)=0.01$, $P=0.99$). The data imply that stress and STZ did not affect the motivation or sensorimotor coordination of the animals.

Changes in LRP1 and RAGE mRNA

LRP1 and RAGE mRNA expression were analyzed in the hippocampus of rats by real-time PCR. The expression levels of LRP1 and RAGE in the stress group before ICV injection of STZ are indicated in Figure 4. Figure 4A shows the mRNA levels of LRP1 in the hippocampus of rats. One-way ANOVA revealed a significant difference between groups ($F(3, 8)=356.7$, $P<0.001$). Post-hoc Tukey's test showed that the mRNA levels of LRP1 were decreased in Stress, STZ, and Stress+STZ groups compared with the sham group. Interestingly, this reduction was severe when animals received stress prior to STZ. Figure 4B shows the mRNA levels of RAGE in the hippocampus of rats. One-way ANOVA revealed a significant difference between groups ($F(3, 8)=58.61$, $P<0.001$). On the other hand, although stress, STZ, or Stress+STZ increased the mRNA levels of hippocampal RAGE, this enhancement was much more significant in the Stress+STZ group.

Result of Stress group after ICV injection of STZ

Body weight difference

As shown in figure 5, changes in body weight in the stress group after ICV injection of STZ were similar to that of the stress group before ICV injection of STZ. In other words, BWD significantly was reduced in the STZ and STZ+Stress groups compared to that of the sham and stress groups ($P<0.05$).

Spatial learning and memory

Figure 6 also shows the performance of the stress group after ICV injection of STZ in the MWM test. Figure 6A shows animals' learning ability during three consecutive days of training. The repeated measure analysis showed a significant difference in escape latency between the groups ($F(3, 24)=20.48$, $P<0.01$). Post-hoc Tukey's test showed that the mean escape latency in STZ groups (with or without stress) is significantly greater than sham and stress groups. Figure 6B shows the frequency of animals' entrance into the platform area and its proximity. One-way ANOVA revealed a significant difference between groups ($F(3, 24)=6.8$, $P<0.01$). Post-hoc Tukey's test showed that the frequency of entry into the platform area and its proximity STZ+Stress and STZ groups is significantly different from the sham group. Figure 6C shows the time spent in the platform area and its proximity. One-way ANOVA revealed a significant difference between groups ($F(3, 24)=51.3$, $P<0.01$). Post-hoc Tukey's test showed that the time spent in the platform area and its proximity in Stress and STZ groups (with or without stress) is significantly different from sham and stress groups. Figure 6D shows the mean swimming velocity during days of training. One-way ANOVA of swimming speed did not show significant differences between groups ($F(3, 24)=1.67$, $P=0.2$), which means that the animal's performance was not affected by the swimming speed. Figure 6E shows the escape latency to reach the visible platform. One-way ANOVA test on the escape latency to the visible platform did not show significant differences between groups ($F(3, 24)=0.04$, $P=0.99$). This finding suggests that stress and STZ did not affect animals' motivation or sensorimotor coordination.

Changes in LRP1 and RAGE mRNA

The expression levels of LRP1 and RAGE in the stress group after ICV injection of STZ are indicated in Figure 7. Figure 7A shows the mRNA levels of LRP1 in the hippocampus of rats. One-way ANOVA revealed a significant difference between groups ($F(3, 8)=255.9$, $P<0.01$). Post-hoc Tukey's test showed that the mRNA levels of LRP1 were decreased in Stress, STZ, and STZ+Stress groups compared with the sham group. Interestingly, this reduction was much less in the STZ+stress group. Figure 7B shows the mRNA levels of RAGE in the hippocampus of rats. One-way ANOVA revealed a significant difference between groups ($F(3, 8)=48.99$, $P<0.001$). Post-hoc Tukey's test showed that the hippocampal RAGE mRNA levels were significantly upregulated in the STZ and Stress groups compared to the sham group. In addition, the mRNA levels of RAGE were increased in STZ+Stress groups compared with other groups.

Discussion

The present research evaluated the impact of chronic electric foot shock stress (before and after bilateral ICV-STZ injection) on body weight, spatial learning, memory, and hippocampal LRP1 and RAGE expression in a rat model of AD. According to the outputs, exposure to 10-day chronic electric foot shock stress decreased body weight, impaired spatial learning, and memory declined hippocampal LRP1 mRNA expression and enhanced hippocampal RAGE mRNA expression in the rat model of AD.

Weight loss and malnutrition have been identified as possible consequences of AD in studies. Additionally, weight loss is associated with rapid memory loss in patients with AD, emphasizing the importance of addressing weight loss and malnutrition in these patients (Gillette-Guyonnet et al., 2000; Kimura et al., 2019). Previously published research established that accumulating A β in the brain would impair the body's weight regulation mechanism; thus, resulting in increased weight loss years before AD diagnosis (Rabin et al., 2020). Further analyses revealed that ICV-STZ would cause syndromes mimicking the sporadic AD in the rats and thus produce an imbalance in the cerebral energy and declining body weight (K Paidi et al., 2015). The present research found animal weight loss following STZ injection into ventricles, similar to the observations reported by other researchers (K Paidi et al., 2015; Khalili & Hamzeh, 2010). On the other hand, stress has been shown to alter body weight and food intake in animal models (Geiker et al., 2018; Moreira, Almeida, Leite-Almeida, Sousa, & Costa, 2016). Consistent with our findings, multiple studies have demonstrated that chronic stress has no discernible effect on body weight compared to controls (Li, Zhang, & Huang, 2009; Liao et al., 2013). At the same time, several studies have found that chronic exposure to certain types of stressors causes either a decrease (Jeong, Lee, & Kang, 2013; Quan et al., 2011) or an increase (Geiker et al., 2018; Scott, Melhorn, & Sakai, 2012) in body weight. Diverse outputs may result from stress intensity, type, or duration variations. Intriguingly, our study found that rats lost a significant amount of weight following the accumulation of electric foot shock stress and STZ administration.

According to the studies in the field, learning and memory deficits have been proposed as the prime issues in AD, which prevent patients from enjoying everyday life (Jahn, 2013; White & Ruske, 2002). The results of our study and previous investigations confirmed a lower level of spatial learning and memory in the ICV-STZ animals on MWM (K Paidi et al., 2015; Negintaji et al., 2015; Sasaki-Hamada, Ikeda, & Oka,

2019). On the other hand, chronic stress would stimulate the enhanced levels of glucocorticoid stress hormones, which have detrimental impacts on the function and structure of the CNS, particularly the hippocampus (Conrad, 2010; Shors, 2004). We showed that chronic electric foot shock for ten days induced memory impairment in normal rats that matched earlier findings (Moosavi, Naghdi, Maghsoudi, & Asl, 2007; Wright & Conrad, 2008). Although there is insufficient information on the etiology of more common (sporadic) forms of AD, previous research indicated that the interaction of environmental risk factors and genetic backgrounds plays a significantly influential role in the onset and progression of sporadic AD. Epidemiological studies have identified stress as a risk factor for AD. The evidence suggested that AD would impair the normal functioning of the hypothalamic-pituitary-adrenal axis. The enhanced level of glucocorticoid can hit the hippocampus due to high levels of glucocorticoid receptors in the hippocampus of its neurons. Earlier research indicated that glucocorticoids and stress increased APP, BACE, and C99 levels, implying that stress induces APP processing along the amyloidogenic pathway, resulting in increased plaque formation and accelerating the neuropathology of AD, including increased A β deposition in the hippocampus (Cuadrado-Tejedor et al., 2012; Dong & Csernansky, 2009; Green, Billings, Roozendaal, McGaugh, & LaFerla, 2006). Also, studies conducted during the last decade found a relationship between levels of amyloid deposition and memory performance (Ford et al., 2015; Ramírez, Mendieta, Flores, & Limón, 2018). Hence, diverse spatial tasks have been utilized for assessing hippocampal functions, and MWM has been introduced as one of the classical tests of spatial learning and memory for rodents. A majority of the MWM investigations reported that chronic stress impaired spatial memory in the rat model of AD (Conrad, 2010; Vorhees & Williams, 2006). What was clear based on our findings is a considerable decline in spatial learning and memory when STZ coincided with chronic stress.

There is a correlation between neurological disorders, cerebrovascular dysfunction, and BBB function changes (Montagne, Zhao, & Zlokovic, 2017). Moving A β across BBB needs a professional transport system. Notably, LRP1 and RAGE receptors contribute significantly to the free un-bound A β between blood and brain and across BBB (Deane, 2012; Sagare et al., 2012). Moreover, neurodegenerative illnesses like AD showed more significant levels of RAGE and lower levels of LRP1 related to the A β toxicity-induced damages on the hippocampal cells (Erickson & Banks, 2013). Li Wang et al.'s (2017) study showed enhancement of RAGE and diminishment of LRP1 at BBB in the STZ-induced rat model of AD (L. Wang et al., 2017). In this sense, the present research confirmed the results obtained from earlier experiments. On the other hand, some investigations studied the impact of chronic and acute stress on the BBB functions (Kempuraj et al., 2019). A limited number of studies reported the correlation between chronic stress and BBB transport system dysfunction markers (Kempuraj et al., 2019). Our research revealed chronic electric foot-shock stress-induced lower LRP1 expression and greater RAGE expression in the rats' hippocampus. Based on the results of the previous studies, stress can exacerbate other disorders like depression (Plieger, Melchers, Montag, Meermann, & Reuter, 2015), metabolic syndrome (Tamashiro, Sakai, Shively, Karatsoreos, & Reagan, 2011), and diabetes (Pavlatou et al., 2008). Previous research has indicated that RAGE increased and LRP1 decreased at the BBB of STZ-induced diabetic rats. The results revealed that the upregulation of RAGE and downregulation of LRP1 expression at the BBB

contribute to A β deposition in diabetes mellitus (Hong et al., 2009; Liu et al., 2009). Additionally, Franklin et al. (2018) found that chronic unpredictable stress increased the mRNA levels of RAGE and high mobility group box one protein (HMGB1) in enriched hippocampal microglia. Furthermore, they demonstrated that RAGE deletion mutant mice are resistant to chronic unpredictable stress-induced behavioral deficits (Franklin et al., 2018). On the other hand, Wang et al. (2019) evidenced that chronic unpredictable mild stress increased hippocampal LRP1 expression in a depressive-like adult male rat model (H. Wang, Xiao, Wang, & Wang, 2020). The current research findings confirmed that electrical foot shock stress decreases LRP1 expression. The differences could be attributed to various factors, including the stress paradigm type and timing of stressors) used in the studies and the methodology used.

Conclusion

According to mounting evidence, chronic stress exposure is a risk factor for people with AD, adversely affecting the disease's course. In previous studies, chronic stress has been shown to sensitize brain cells to subsequent challenges, and severe stress elicits synergistic toxicity in response to a second challenge. In other words, subsequent exposure of the preconditioned organism to stress stimuli can increase the brain's susceptibility to AD. Investigating the underlying mechanisms of stress-induced brain vulnerability provides a foundation for understanding the relationship between the efficacy of stress interventions and neurodegenerative disorders such as Alzheimer-related memory decline. According to our findings, stress prior to and following STZ ICV injection exacerbates LRP1 reduction and RAGE enhancement in the hippocampus. The study results pave a new way for scientists to develop novel strategies for AD treatment.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interests.

Authors' contributions

Conceptualization [AZ, HA, VR]; Methodology [AZ, HA, VR]; Investigation [ZT]; Writing – Original Draft [ZT, HA]; Writing – Review & Editing [all authors]; Funding Acquisition [HA]; Resources [HA]; Supervision [AZ, HA, VR].

Ethical Considerations

Compliance with ethical guidelines

The experimental protocols were approved by the ethics committee of Shiraz University of Medical Sciences and the animal care was according to the NIH Guide for the care and use of laboratory animals. In this study, all efforts were made to minimize the number of animals used and their suffering.

Data availability

Data will be made available on reasonable request

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Figures

Figure 1

Experimental timeline of the study including STZ-injection, chronic stress exposure, behavioral testing, and tissue collection. A: Stress group before ICV injection of STZ. B: Stress group after ICV injection of STZ.

Figure 2

Comparison of body weight differences (BWD) in stress group before ICV injection of STZ. Data are represented as mean $\pm$ S.E.M (n=7). **p<0.01 compared with control and sham groups and #P < 0.05 and ##P < 0.01 compared with stress group.

Figure 3

The performance of the stress group before ICV injection of STZ in the MWM test. A: The mean escape latency to reach the hidden platform. Data are represented as mean $\pm$ S.E.M (n=7). **p<0.01 indicates the significant difference between the groups. B: The frequency of entrance into the target zone. **p<0.01 compared with the sham group and #P < 0.05 compared with stress group. C: The time spent in the target quadrant. *p<0.05 and **p<0.01 compared with sham group and ##P < 0.01 compared with stress group. D: the mean swimming velocity. E: the mean escape latency to reach the visible platform.

A

B

Figure 4

The expression levels of LRP1 and RAGE in the stress group before ICV injection of STZ. A: Comparison of changes in LRP1 mRNA expression level in all groups. Data are represented as mean $\pm$ S.E.M. ** $p < 0.01$ compared with the sham group and ## $P < 0.01$ compared with stress group and + $P < 0.01$ compared with STZ group. B: Comparison of changes in RAGE mRNA expression level in all groups. * $p < 0.05$ and ** $p < 0.01$ compared with sham group and ## $P < 0.01$ compared with stress group.

Figure 5

Comparison of body weight differences (BWD) in stress group after ICV injection of STZ. Data are represented as mean $\pm$ S.E.M (n=7). **p<0.01 compared with control and sham groups and #P < 0.05 and ##P < 0.01 compared with stress group.

Figure 6

The performance of the stress group after ICV injection of STZ in the MWM test. A: The mean escape latency to reach the hidden platform. Data are represented as mean $\pm$ S.E.M (n=7). **p<0.01 indicates the significant difference between the groups. B: The frequency of entrance into the target zone. **p<0.01

compared with the sham group. C: The time spent in the target quadrant. $**p < 0.01$ compared with sham group and $##P < 0.01$ compared with stress group. D: The mean swimming velocity. E: The mean escape latency to reach the visible platform.

Figure 7

The expression levels of LRP1 and RAGE in the stress group after ICV injection of STZ. A: Comparison of changes in LRP1 mRNA expression level in all groups. Data are represented as mean $\pm$ S.E.M. $**p < 0.01$ compared with sham group and $##P < 0.01$ compared with stress group and $++P < 0.01$ compared with STZ group. B: Comparison of changes in RAGE mRNA expression level in all groups. $**p < 0.01$ compared with sham group and $\#P < 0.05$ and $##P < 0.01$ compared with stress group $+P < 0.01$ compared with STZ group.