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Original Article

Gap junctions of the hippocampal CA_1 area are crucial for memory consolidation

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Abstract

Introduction: Gap junctions are specialized cell–cell contacts between eukaryotic cells through which they communicate. This type of communication has the potential to modulate memory process. We evaluated the effects of the gating of the hippocampal CA_1 area gap junction channels on memory consolidation, using passive avoidance task.

Materials and Methods: 72 adult male Wistar rats were distributed into 9 groups of 8 each. Two guiding cannulas were bilaterally implanted in the hippocampal CA_1 area of all rats. One week after surgery, the animals received an electrical shock with the intensity and duration of 0.3 mA and 1s, respectively. Immediately after training 25, 75 or 150 nM doses of carbenoxolone, a non-selective blocker of gap junction channels or 50, 150 and 1500 nM doses of trimethylamine, an opener of gap junction channels were injected. Another group received 50 nM trimethylamine and 10 min later 75 nM carbenoxolone, immediately post-training. 24 hours later, memory retrieval was assessed.

Results: Post-training injection of carbenoxolone significantly and dose- dependently decreased step-through latency, whereas post-training injection of trimethylamine showed a tendency toward increasing step-through latency. Post-training injection of trimethylamine (50 nM) increased step-through latency, significantly compared with post-training injection of carbenoxolone (75 nM and 150 nM). Post-training injection of trimethylamine (50 nM) before carbenoxolone (75 nM) reversed the effects of carbenoxolone on inhibition of memory consolidation.

Conclusion: These data suggest that the intercellular coupling via gap junction channels in the hippocampal CA_1 cells is crucial for memory consolidation in the passive avoidance task.

Keywords:
Gap junction;
Memory;
Hippocampal CA_1 area;
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Introduction

Gap junctions are specialized cell–cell contacts between eukaryotic cells, composed of aggregates of trans-membrane channels (Dere and Zlomuzica, 2012). These channels couple metabolic and transcriptional activities between adjacent cells. Gap junctions also act in neuronal networks to coordinate cell firing (Willecke et al., 2002). Although gap junctions are expressed throughout the nervous system, their functional importance has been underestimated. Studies have

suggested that intercellular communication via gap junction channels modulate learning and memory processes (Dere and Zlomuzica, 2012). Memory formation consists of several stages including acquisition, consolidation, retention and retrieval (Abel and Lattal, 2001).

The hippocampus is an important area in the brain for controlling learning and memory (Zola-Morgan et al., 1986). It is well known that different cell types within the hippocampus communicate with each other via gap junctions (Sohl et al., 2005). The CA_1 area of the hippocampus is involved in consolidation of memory in the passive avoidance response (Lorenzini et al., 1996). It was shown that blocking neuronal gap junctions within the dorsal hippocampus impaired context-dependent fear learning, memory and extinction (Bissiere et al., 2011). Chepkova et al indicated that carbenoxolone impaired LTP in murine hippocampus (Chepkova et al., 2008). Furthermore, blocking gap junction channels of the hippocampal CA_1 area by carbenoxolone modulated morphine state dependent learning (Beheshti et al., 2014). However, the effect of gating of gap junction channels in the CA_1 area of the hippocampus on memory consolidation in the passive avoidance paradigm has not been studied.

There are many agents who can close or open the gap junction channels (Salameh and Dhein, 2005). Carbenoxolone is a gap junction blocker that has been used to study the involvement of gap junctions in intercellular interactions. It is a non-selective blocker of connexin proteins that form gap junctions (Herve and Sarrouilhe, 2005). Trimethylamine opens gap junction channels by causing intracellular alkalization (Gajda et al., 2003).

Though the results of most studies have indicated a relationship between gap junctions and memory formation (Hosseinzadeh et al., 2005; Chepkova et al., 2008; Allen et al., 2011; Bissiere et al., 2011; Beheshti et al., 2014), there are rare reports indicating the role of gap junctions for each of the particular stages of memory formation. In the present study, we evaluated the effects of either closing or opening of the hippocampal CA_1 area gap junction channels on memory consolidation via post-training administration of carbenoxolone or trimethylamine, using passive avoidance task in rats.

Materials and methods

Drugs

Carbenoxolone and trimethylamine were purchased from Sigma Company (St. Louis, MO, USA). Ketamine and xylazine were purchased from Alfasan (Netherland). Carbenoxolone and trimethylamine were dissolved in saline. Before the injections, all of the solutions were passed through microfilters (0.22µm, BIOFIL) to be sterilized.

Animals

72 adult male Wistar rats weighing 220±20 g were obtained from the breeding colony of Isfahan University of Medical Sciences and housed at the animal facility of the Department of Biology in standard rat cages. The animal room was maintained on a 12-hour reverse light/dark cycle (lights on at 07:00 P.M.). The room temperature was set at 24º C. For all animals ad libitum access to food and water was available. After the surgery for cannula implantation, each rat was kept in a cage. All experiments were executed in accordance with the policy for the care and use of laboratory animals (National Institute of Health publication No. 80-23, revised 1996) and were approved by the graduate studies committee of Department of Biology, University of Isfahan.

Stereotaxic surgery

Initially, each rat was anaesthetized with a mixture of 100 mg/kg ketamine and 10 mg/kg xylazine administered intraperitoneal. The top of the head was carefully shaved and the rat was placed on the stereotaxic stage (Stoelting, USA). The skin on the head was incised and the bregma was located. Two 22-gauge guide cannulas were bilaterally implanted. They were aimed at 1 mm dorsal to the CA_1 (Anterior-posterior: −3. 36 mm from bregma, midline: ±2. 2 mm, and dorsal-ventral: −2. 8 mm from the skull surface) according to the Rat Brain Atlas (Paxinos, 2007). Two screws were inserted into the skull. The cannulas were fixed to the skull using dental cement paste and closed with stainless steel stylets (27-gauge) smeared with mineral oil to prevent clogging with blood. Each rat was given 7 days to recover from surgery.

Microinjection

Microinjections were performed using 27-gauge needles extended 1 mm from the end of the guide cannula, connected via polyethylene tubing (PE20, Stoelting) to a 2µl Hamilton syringe. The intrahippocampal drug delivery was done bilaterally (0.5 µl/min/side). The injection needles were left in place for an additional minute and then were slowly withdrawn.

Passive avoidance task

The step-through passive avoidance paradigm was performed as previously described (Beheshti et al., 2014). Briefly, each rat was placed in the white chamber of the apparatus facing the sliding door. After 5 s the door was raised. When the animal stepped into the dark chamber with all four paws, the door was closed and the rat remained there for 20 s. Then the animal was removed to be placed in a temporary cage. 30 min later, the rat was again placed in the white chamber for 5 s, then the door was raised to let the animal enter the dark chamber and following entrance, the door was closed, but this time a controlled electrical shock of 0.3 mA lasting for 1 s was delivered. After 20 s, the rat was placed into the temporary cage. 2 min later, the same testing procedure was repeated. The rat received a foot shock each time it stepped into the dark chamber with all four paws. When the rat remained in the white compartment for a 2-min time period, the training was terminated. On the second day, a retrieval test was performed to evaluate long-term memory. Each animal was placed in the white chamber for 20 s, then the door was raised and the time taken to enter the dark chamber called the step-through latency was recorded, up to 600 s.

Experimental Procedures

Experiment 1

This experiment was performed to determine the effects of post-training administration of carbenoxolone or trimethylamine in the hippocampal CA_1 area, on consolidation of learning. Seven groups of animals received saline or the different doses of carbenoxolone (25, 75 and 150 nM) or trimethylamine (50, 150 and 1500 nM) immediately post-training. 24 hours later in the test day, memory retrieval was measured. The doses of the drugs were selected according to previous studies based on their effectiveness on gap junction channel gating (Nassiri-Asl et al., 2008; Beheshti et al., 2014).

Experiment 2

In this experiment, the effects of post-training co-injections of trimethylamine and carbenoxolone in the hippocampal CA_1 area were investigated on memory

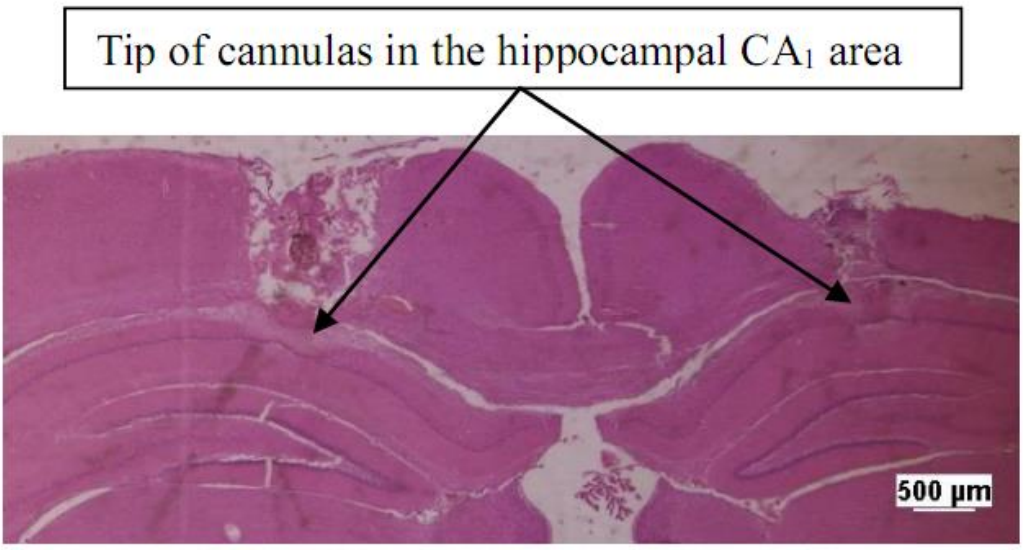

Fig.1. Representative hematoxylin-eosin stained coronal brain section of rats showing the cannula positions in the CA_1 area of carbenoxolone or trimethylamine treated rats.

consolidation. Two groups of animals received effective doses of either trimethylamine (50 nM) immediately post-training and 10 min later carbenoxolone (75 nM) or saline instead of trimethylamine and carbenoxolone. 24 hours later in the test day, memory retrieval was investigated.

Histology

After completion of the experiments, each rat was deeply euthanized with ether in a sealed jar. The brain was removed and kept in 10% formalin for 5 days at room temperature. Then, coronal slices from the site of cannula implantation of the fixed brain were prepared. The brain slices were stained using hematoxylin-eosin procedure. A microscope equipped with a video camera linked to a computer with specific Software was used to take digital images. The cannula positions were verified according to the Rat Brain Atlas (Paxinos, 2007). The data obtained from the who received injections on sites outside of the CA1 area was not used for statistical analysis (Fig. 1).

Statistical analysis

The data are presented as means ± S.E.M. One-way analysis of variance (ANOVA) with Tukey-Kramer multiple comparison post-hoc test or unpaired t-test were performed for data analysis. In all the experiments, $P<0.05$ was considered statistically significant.

Results

The effects of carbenoxolone or trimethylamine on memory consolidation

In the passive avoidance task, the decrease in step-through latency means loss of memory. One-way ANOVA revealed significant changes taking place after measuring the step-through latency following post-training treatments with carbenoxolone ($F=(3, 31)=5.9$; $P=0.0113$; Fig. 2). Post hoc comparison showed that carbenoxolone at doses 75 and 150 nM decreased step-through latency, significantly as compared to control group ($P<0.05$). Post-training injection of trimethylamine showed a tendency toward increasing step-through latency which, failed to reach statistical significance compared with control group (Fig. 3). One-way ANOVA showed significant changes as a result of treatments, with trimethylamine compared with post-training injection of the effective doses of carbenoxolone ($F= (2, 23) = 9.8$; $P= 0.001$; Fig. 4). Post hoc comparison showed that trimethylamine (50 nM) increased step-through latency, significantly compared

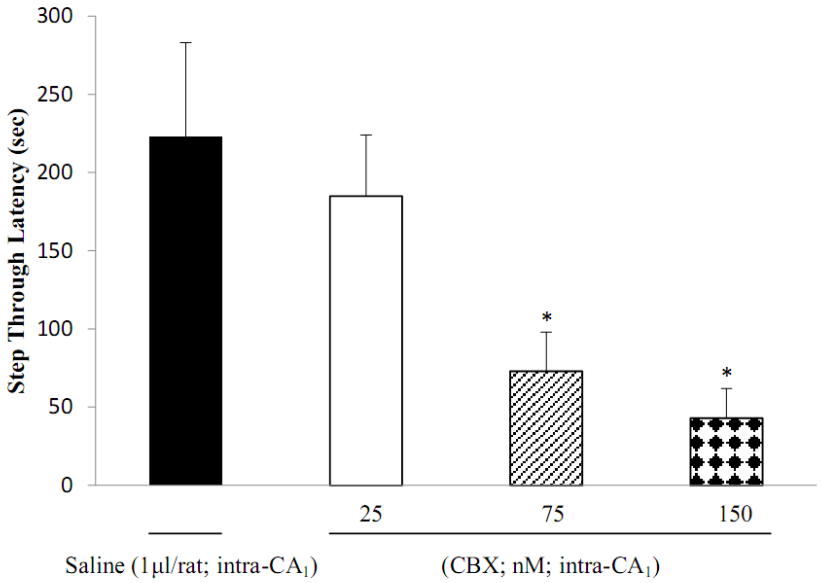

Fig.2. The effect of post-training injection of carbenoxolone (CBX; 25, 75 and 150 nM) in the hippocampal CA_1 area on step-through latency. Data are expressed as means ± S.E.M. (*$P<0.05$).

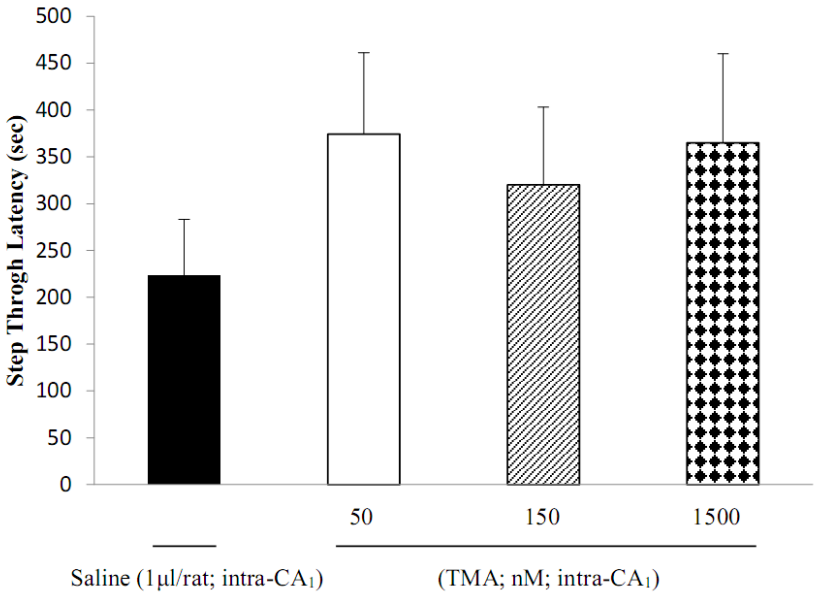

Fig.3. The effect of post-training injection of trimethylamine (TMA; 50, 150 and 1500 nM) in the hippocampal CA_1 area on step-through latency. Data are expressed as means ± S.E.M.

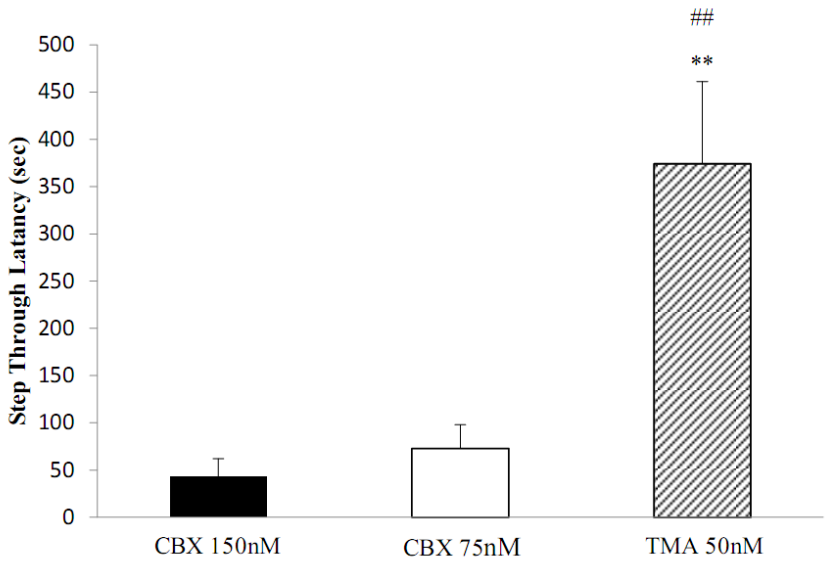

Fig.4. The effect of post-training injection of trimethylamine (50 nM), on increasing step-through latency with respect to post-training injection of carbenoxolone (75 and 150 nM). Data are expressed as means ± S.E.M. (**P<0.01; ##P<0.01 relating to CBX 75 nM or 150 nM, respectively).

with 75nM and 150nM doses of carbenoxolone (P<0.01). Un-paired t-test indicated that post-training injection of trimethylamine (50 nM) before carbenoxolone (75 nM), significantly reversed the decreased rate of step-through latency caused by post-training injection of carbenoxolone alone (P=0.04; Fig. 5).

Discussion

The post-training bilateral administration of carbenoxolone in the CA_1 area of the hippocampus impaired memory consolidation in the passive avoidance task. Consolidation is the process of developing a stable memory (Katche et al., 2013).

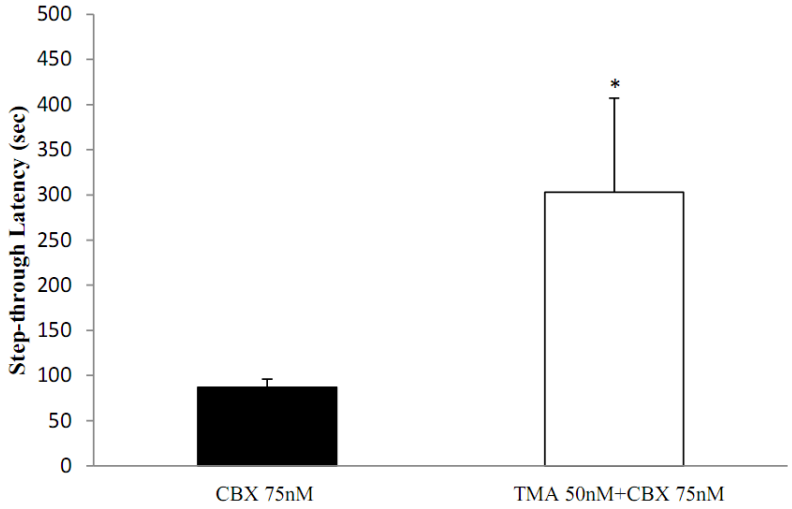

Fig.5. The effect of post-training co-injection of trimethylamine (TMA; 50 nM) and carbenoxolone (CBX; 75 nM) in the hippocampal CA_1 area on step-through latency. Data are expressed as means ± S.E.M. (*P<0.05).

Following data acquisition, consolidation is achieved when the brain areas involved in memory processing are altered. For memory processing, activities across distinct groups of neurons are required to be coordinated. Synchronization of neuronal activity by oscillation is one of the mechanisms utilized this process (Colgin and Moser, 2010). There are growing numbers of evidence indicating that gamma, theta and high frequency oscillatory activities in the brain are involved in various stages of memory process including consolidation (Duzel et al., 2010; Hanslmayr and Staudigl, 2014). In the hippocampus, coordination of cell firings mediated by gap junctions can generate oscillations of different frequencies (Draguhn et al., 1998; Hormuzdi et al., 2001; Buhl et al., 2003; Konopacki et al., 2004).

An in vitro study using hippocampal slices indicated that blocking chemical synaptic transmission maintains high frequency oscillations or ripples intact, whereas gap junction uncouplers block these oscillations (Draguhn et al., 1998). During hippocampal ripples, a powerful synchronization system connects the neuronal networks of the hippocampus to that of neocortex. This connection was suggested to be involved in memory consolidation (Chrobak and Buzsaki, 1996). Furthermore, carbenoxolone could decrease synchronization of an interneuron network of gamma oscillations in vitro (Traub et al., 2001). It was indicated that gamma oscillations connect the CA_1 area of the hippocampus with the central area of the entorhinal cortex and this connection was involved in memory consolidation (Colgin and Moser, 2010). Carbenoxolone could also reversibly block generation of hippocampal theta oscillations recorded in the slice preparations and urethane anaesthetized rats (Bocian et al., 2009). Therefore, carbenoxolone might suppress ripples, gamma and theta oscillations through blocking gap junction channels, inhibiting the widespread activation of hippocampal targets.

Injection of carbenoxolone in the rat dorsal hippocampus was shown to impair the consolidation of contextual fear memories (Bissiere et al., 2011). Also, bilateral administration of carbenoxolone in the CA_1 area of the rat hippocampus inhibited memory consolidation as demonstrated by the Morris water maze (Hosseinzadeh et al., 2005). These reports support our results.

It was reported that carbenoxolone has a number of other effects, including a reduction in excitatory and inhibitory synaptic currents, alteration of intrinsic membrane properties and suppression of action potentials (Rekling et al., 2000; Rouach et al., 2003; Tovar et al., 2009). Therefore, we aimed to examine whether the effects of carbenoxolone on impairment of memory consolidation could be due to uncoupling of gap junctions. For this purpose, we assessed the co-injection effects of trimethylamine and carbenoxolone in the hippocampal CA_1 area on memory consolidation. Post-training administration of trimethylamine before carbenoxolone could reverse the

inhibition of memory consolidation caused by post-training injection of carbenoxolone alone. Accordingly, the reason that carbenoxolone inhibits memory consolidation could be mainly due to the stalling the intercellular communications via hippocampal CA_1 area gap junctions.

The results of this study also indicated that post-training bilateral administration of trimethylamine in the rat hippocampal CA_1 area, improved memory consolidation. The opening of gap junction channels influenced by trimethylamine may have increased neural oscillatory functions in the CA_1 area, leading to improved memory consolidation. To support this hypothesis, it was indicated that trimethylamine increased amplitude and power of theta oscillations in the hippocampus (Bocian et al., 2011). Post-training injection of trimethylamine (50 nM) increased step-through latency, significantly compared with post-training injections of the effective doses of carbenoxolone (75 and 150 nM). This shows that depending on state of the CA_1 area gap junction channels, memory parameters change profoundly.

Gap junction plaques contain many intercellular channels, of which some of are closed while the others are open at any given time. Gap junction channel blockers or openers modify the percentage of either the open or the closed channels (Rozental, 2001). Based on the results of this study, we hypothesize that during memory consolidation, the percentage of the opened channels in the hippocampal CA_1 area might have increased. Thus, when trimethylamine was injected, the number of open channels was not severely altered with respect to the specific physiological conditions. On the other hand, carbenoxolone could block the majority of gap junction channels (Rozental, 2001). Consequently, the effect of carbenoxolone on memory consolidation was more prominent than those of trimethylamine. Taken together, as carbenoxolone could impair memory consolidation, it appears that opening state of the hippocampal gap junction channels is required for this particular stage of memory formation in the step-through passive avoidance task.

In conclusion, the results of the present study indicate that opening and closing state of the hippocampal CA_1 area gap junction channels could affect memory consolidation in the passive avoidance paradigm. The intercellular coupling via gap junction channels between hippocampal CA_1 area neural cells was indicated to be crucial for memory consolidation.

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Conflict of interest

None

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