Research Report

The role of GABAergic transmission in the dentate gyrus on acquisition, consolidation and retrieval of an inhibitory avoidance learning and memory task in the rat

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ARTICLE INFO

Article history:
Accepted 3 February 2008
Available online 14 February 2008

Keywords:
Picrotoxin
Dentate gyrus
Inhibitory avoidance learning
Rat

ABSTRACT

The hippocampal GABAergic interneurons are responsible for controlling the input of large principal cell populations, and thereby determine the oscillatory discharge patterns and synaptic plasticity in the hippocampus. Such oscillations within neuronal systems serve various complex functions, such as perception, cognition, plasticity and memory. The aim of this study is to define the function of GABAergic synaptic transmission in the dentate gyrus (DG) of the hippocampus in the different stages of inhibitory avoidance (IA) learning and memory in the rat. Two cannulae were implanted above the hippocampal DG. Then the rats were trained on a step-through IA learning task. Each rat received intra-DG injection of picrotoxin (PTX) or saline before training, after training or before the retrieval test. The results show that post-training injection of PTX impaired the IA memory. On the other hand, pre-training and pre-retrieval injection of PTX had no significant effect on the IA activity. Therefore, it seems that GABAergic transmission in the DG is involved in the consolidation step (but not in the acquisition and retrieval steps) of the IA task by controlling the input to the principal cells.

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1. Introduction

Learning and memory are complex processes (Das, 2003; Markowitsch, 1997; Fuster, 1998; Goldman-Rakic, 1996; Lu and Figurov, 1997) that have several stages, including acquisition, consolidation and retrieval (Okano et al., 2000; Abel and Matthew, 2001). A number of neurotransmitters are in some way involved in the formation and retrieval of memory traces (Das, 2003; Farr et al., 2000). The GABAergic system is critically involved in cognitive processes, especially learning and memory (Castellano et al, 1993; Chapouthier and Venault, 2002; Garden et al, 2002; Tosso et al, 2007; Berlau and McGaugh, 2006; Zarrindast et al, 2006; Lee et al, 2006; Ramirez et al, 2005; Jiang et al, 2005; Collinson et al 2002; Zarrindast et al, 2004). Previous studies have
shown that compounds which activate the GABAergic system also impair memory formation. Conversely, drugs that decrease GABA function can have memory-enhancing properties (Nutt and Malizia, 2001; Brioni and McGaugh, 1988; Brioni et al., 1989; Castellano and McGaugh, 1990; Tomaz et al, 1993; Farkas and Crowe, 2000).

In the hippocampus, which is widely recognized as an important neural structure in learning and memory (Suzuki, 2007; Martin and Clark, 2007; Sutherland et al, 2006; Buckley, 2005; Vianna et al, 2004; Mizuno and Giese 2005; Eichenbaum, 2004; Tulving and Markowitsch, 1998), all excitatory inputs have their private specialized class of interneuron (Buzsáki, 2001; Freund et al, 1990; Freund and Antal, 1988; Miettinen and Freund, 1992). In other words, in the hippocampus, several GABAergic inhibitory interneuron types have been identified that form synapses on different domains of their postsynaptic target cells (Han et al., 1993; Freund and Buzsáki, 1996). For example, in the dentate gyrus (DG), basket cells (interneurons that innervate the perisomatic domain of principal neurons) mediate a particularly powerful form of inhibition (Miles et al., 1996).

From a physiological point of view, the role of the GABAergic inhibitory interneurons of the hippocampal DG in the different steps of learning and memory is not clearly understood. The aim of this study is to describe the current state of knowledge about the function of GABAergic synaptic transmission in the DG of the rat. GABA receptors (GABA-A and GABA-B receptors) participate in the functional circuits of the DG and could control the excitability of principal cells (Piguet, 1993). As GABA-A receptors are more prominent than GABA-B receptors in the DG, we have chosen to evaluate the effects of intra-DG injection of a GABA-A receptor antagonist, picrotoxin (PTX). Specifically, we examine its ability to facilitate or impair the acquisition, consolidation and retention of an inhibitory avoidance (IA) task.

2. Results

2.1. Experiment 1. Effects of pre-training intra-DG injection of PTX and saline on acquisition of IA task

The results of Experiment 1 indicate that PTX had no effect on the acquisition of the IA task. A Student’s t-test indicates that before the acquisition trial there was no significant difference between the saline and PTX groups in the Step-through latency (STL) ($t_{[15]}=0.39, p>0.05$) (Fig. 1A). This result reveals that intra-DG infusion of PTX had no significant effect on the exploratory behavior of rat to the dark. Also, a Student’s t-test shows that there is no significant difference in the number of trials to acquisition criterion ($t_{[15]}=0.64, p>0.05$) (Fig. 1B). Therefore, pre-training intra-DG administration of PTX had no effect on the acquisition.

2.2. Experiment 2. Effects of post-training intra-DG injection of PTX and saline on consolidation of IA task

A Student’s t-test indicates that before the acquisition trial there was no significant difference between the saline and PTX groups in the STL of first acquisition trial ($t_{[17]}=0.64, p>0.05$). Also, a Student’s t-test shows that there is no significant difference in the number of trials to acquisition criterion ($t_{[17]}=0.46, p>0.05$). Therefore, both groups of rats showed the same behavior before receiving PTX or saline.
In the retention test, which was performed 24 h later, there was a significant difference between these groups. Statistical analysis using a Student’s t-test showed that the STL in the PTX treated group was significantly less than the STL in the saline treated group ($t_{[17]}=2.44, p<0.05$) (Fig. 2A). The percent of time spent in the dark compartment (TDC) (after first stepping into it) by the PTX treated group was significantly more than that spent by the saline treated group ($t_{[17]}=2.44, p<0.05$) (Fig. 2B). These results demonstrate that a post-training intra-DG infusion of PTX impaired the consolidation stage of IA memory.

2.3. Experiment 3. Effects of pre-retrieval intra-DG injection of PTX and saline on retention of IA task

There was no significant difference between the saline and PTX groups in the STL of first acquisition trial ($t_{[16]}=0.41, p>0.05$). Also, a Student’s t-test showed that there is no significant difference in the number of trials to acquisition ($t_{[16]}=0.85, p>0.05$). Therefore, these two groups were uniform before the retention test.

In the retention test, which was again performed 24 h later, we found no significant difference between the two groups in the STL ($t_{[16]}=0.29, p>0.05$) or in the percent of time spent in the dark compartment (TDC) after first stepping into it ($t_{[16]}=0.46, p>0.05$) (Fig. 3). This means that PTX had no significant effect on the retrieval of IA memory.

3. Discussion

In the current study, we investigated the effects of GABA-A mediated inhibition in the DG of the hippocampus on three stages of an IA task. These results showed a selective involvement of these GABA-A receptor in learning and memory processes. Intra-DG injection of PTX immediately after training impaired consolidation of the IA task. Therefore, we conclude that the GABA-A receptors play a facilitatory role in the consolidation of the IA task, but had no significant effect on the acquisition or retention of the task.

GABA is one of the main inhibitory neurotransmitters in the nervous system. It opens chloride channels in a cell by mediating its GABA-A receptors and thus hyperpolarizes the cell (Barnard et al, 1998; Costa, 1998). PTX inhibits these ligand-gated chloride channels (Bormann, 1988; Macdonald and Olsen, 1994).

There is a great deal of evidence supporting the involvement of the GABA-A receptor in learning and memory processes. Administration of compounds that enhance the action of GABA, such as benzodiazepines and muscimol, impair memory processing. On the other hand, compounds which reduce the action of GABA, such as bicuculline or PTX, enhance memory processing or long term potentiation (LTP) induction (Yonkov and Georgiev, 1985; Brioni and McGaugh, 1988; Brown et al, 1988; Brioni et al, 1989; Castellano and McGaugh, 1990; Nagahara and McGaugh, 1992; Tomaz et al, 1993; Clements and Bourne, 1996; Chapouthier and Venault, 2002; Komaki et al, 2007).

It has been reported that a potential role of hippocampal GABAergic interneurons is to provide spatial and temporal conditions for the modification of synaptic weights during hippocampus-dependent memory processes (Paulsen and Moser, 1998). Pre-training, post-training or pre-retrieval inactivation of the dorsal and ventral hippocampus impairs the acquisition, consolidation and retrieval of an IA task in the rat (Ambroggi Lorenzini et al, 1996, 1997). This reveals that all three steps of the IA task processing are hippocampus-dependent.

Our results show that post-training injection of PTX impairs IA, whereas pre-training and pre-retrieval injections of PTX have no significant effect on IA activity. We therefore conclude that dentate gyrus GABAergic transmission is active in the consolidation step of the IA task and may control the output to the principal cells. It is possible that GABAergic transmission does not have a functional role during the acquisition and retention steps of IA task. In this respect, our results are in contrast with the previously mentioned reports. This controversy may be explained by considering other studies about the role of septohippocampal pathway on the hippocampal activity. It has been reported that septohippocampal GABA neurons selectively innervate only the GABA interneurons in the hippocampus (Freund and Antal, 1988; Miettinen and Freund, 1992). The septohippocampal cholinergic neurons provide an excitatory drive to the septohippocampal GABA pathway and inhibit hippocampal GABAergic neurons (and hence disinhibit the principal neurons) (Freund and Antal, 1988; Toth et al, 1997). This effect would facilitate the induction of LTP (Pavlides et al, 1988).

The medial septum plays an important role in the consolidation (but not the acquisition and retrieval) of the IA task and in the induction of LTP in the hippocampal DG (Rashidy-Pour et al, 1995, 1996). Therefore, it is possible that in the consolidation step of the IA task information is relayed to the hippocampus to release GABA on the inhibitory interneurons in the DG, and that this reaction causes disinhibition and facilitates the consolidation of information in memory. In the present study, an intra-DG injection of PTX prevented the septohippocampal inhibition of interneurons and therefore
resulted in an increase of their activity. Thus, interneurons released more GABA onto the principal neurons. Although GABA-A receptors on the principal cells were blocked by PTX, these principal neurons could have still been inhibited because GABA-B receptors still exist on the principal neurons and could control the excitability of these neurons in the DG (Canning and Leung, 2000). However, it should be noted that in the DG, interneurons that innervate the perisomatic domain of principal neurons mediate a particularly powerful form of inhibition (Miles et al., 1996). Thus, PTX inhibited interneurons more than the principal cell. These changes, in turn, decreased the induction of LTP in the DG principal neurons and impaired the consolidation of the IA task.

A comparison of the present result with the previously mentioned studies reveals that GABAergic transmission in the DG of the hippocampus has a different role here than it does in other brain areas. Such inconsistencies are not entirely new. For example, PTX has been found to potentiate LTP induction in the somatosensory cortex (Komaki et al., 2007) and inhibit it in the motor cortex (Trepel and Racine, 2000). The most prominent explanation for this opposite effect was the difference in location.

Finally, we can conclude that during the memorization process of the IA task, GABAergic transmission in the DG of the hippocampus has a different role than it does in other brain areas, and it could facilitate the process of consolidating the IA task.
4. **Experimental procedures**

4.1. **Animals**

Adult male Wistar rats weighing 200–250 g were used. They were obtained from a colony of Iran Pasteur Institutes. They were housed 3 per cage and maintained on a 12–12 h light/dark cycle (lights on at 7:00). Food and water were available ad libitum.

4.2. **Surgery**

Approximately two weeks prior to initiation of the behavioral experiments, the rats were anesthetized with a mixture of ketamin (100 mg/kg) and xylazine (2.5 mg/kg) and were implanted with two cannulae bilaterally (15 mm, 23-gauge) (Shahidi et al, 2004a,b) aimed at a site 1 mm above the DG (AP: −3.8 mm, L: 1.4 mm, DV: −2.8, from skull surface) according to the atlas of Paxinos and Watson (2005) (Fig. 4). The cannulae and two anchoring screws were fixed to the skull with dental cement. The cannulae were closed with styletes.

4.3. **Microinjection**

Before injection, the animal was restrained by hand and the cannulae stylet was removed and replaced with the injection needle (30-gauge) connected with a short piece of polyethylene tubing to a Hamilton syringe. The needle was inserted 1 mm beyond the tip of the cannula and 0.5 µl of saline or PTX was injected into DG (10 ng/side) for 1 min. The needle was left in place for another 60 s before it was slowly withdrawn. Bilateral infusion processes were done for about 5 min.

4.4. **IA apparatus**

The apparatus and procedure were basically the same as those in our previous studies (Shahidi et al, 2004a; Lashgari et al, 2006). Briefly, the apparatus consisted of a lighted chamber and a dark one. Between the two chambers there was a rectangular opening that could be closed by an opaque guillotine door. The floor of both chambers was made of stainless steel rods. The floor of the dark chamber could be electrified.

The rats were placed in a lighted compartment of the apparatus facing away from the door and, 5 s later, the guillotine door was raised. Once the rats entered the dark compartment, the door was closed and the rats were taken from the dark compartment into their home cage. The habituation trial was repeated after 30 min and followed (after the same interval) by the first acquisition trial. Entrance latency to the dark compartment STL was recorded when the animal had placed all 4 paws in the dark compartment. After the animal had spontaneously entered the dark compartment, the guillotine door was lowered and a mild electrical shock (0.6 mA) was applied for 3 s. The rat was retained in the apparatus and received a foot shock each time it re-entered the dark compartment. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds.

The retention test was performed 24 h after the IA task acquisition trial. The rat was placed in a lighted chamber as in the IA task training and, 5 s later, the guillotine door was raised. Then the STL and the percent of TDC after first stepping in were recorded up to 300 s. If the rat did not enter the dark compartment within 300 s, the retention test was terminated and a ceiling score of 300 s was assigned. The timeline of the study is indicated in the Fig. 5.

4.5. **Experimental protocol**

4.5.1. **Experiment 1**

The aim of this experiment was to determine the effect of intra-DG GABAergic blocking on the PA acquisition. The rats were divided into two experimental groups, Saline (n=11) and PTX (n=6) treated, and received intra-DG injection of saline or PTX 5 min before the acquisition trial. The STL of first acquisition trial and the number of trials to PA acquisition were recorded.

4.5.2. **Experiment 2**

The aim of this experiment was to determine the effect of intra-DG GABAergic blocking on the PA consolidation. The rats were again divided into two experimental groups, Saline (n=12) and PTX (n=7) treated. After receiving shock in the dark compartment and returning to the light compartment for 120 consecutive seconds, the rat was removed from the apparatus and immediately received an intra hippocampal infusion of either saline or PTX. The STL of first acquisition trial, the number of trials to PA acquisition, STL and TDC during the retrieval test were recorded.

**Fig. 5 – The timeline and responses recorded in the study.**

A) The acquisition trials: 1—Infusion of PTX or saline in Experiment 1. 2—Recording the STL of first stepping into the dark compartment. 3—Apply foot shock in the dark compartment. 4—Recording the number of trials to learn the trial task. 5—Infusion of PTX or saline in Experiment 2. B) The retention test which was performed 24 h later (no shock applied): 1—Infusion of PTX or saline in Experiment 3. 2—Recording the STL. 3—Recording the TDC after first stepping in to the dark compartment.
4.5.3. **Experiment 3**

The aim of this experiment was to determine the effect of intra-DG GABAergic blocking on the PA retention test. The rats were divided into two experimental groups, Saline (n=11) and PTX (n=7) treated, and received intra-DG injection of saline or PTX 5 min before the retrieval test. The STL of first acquisition trial, the number of trials to PA acquisition, STL and TDC during the retrieval test were recorded.

4.6. **Histology**

At the end of each experiment rats were deeply anesthetized with ketamine. The brains were fixed by formaline, and then they were sectioned and stained to verify cannulae placements.

4.7. **Statistical analysis**

Statistical comparisons between the saline and PTX treated groups were done by an unpaired. Student’s t-test. All results are shown as the mean±SEM. The level p<0.05 was considered significant.

**Acknowledgments**

This research was supported by grant from the Hamedan University of Medical Sciences. Also, the authors wish to thank DAVID JANGRAW at Columbia University (for revising as a native English speaker and for his excellent comments), Maryam Nourbakhshnia, Monire Akbari Mani and Ranaa Shooshtari for their technical assistance.

**References**


