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Effects of pre-training injection of orexin A into dorsal raphe nucleus in passive avoidance acquisition on male rats

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Abstract

Endogenous orexins, especially orexin A, play an important role in spatial learning and memory. A recent study has shown the effect of orexinergic system in hippocampus on avoidance learning. Orexinergic receptors are distributed in dorsal raphe nucleus (DRN). The aim of this study was to evaluate the role of dorsal raphe orexinergic system in passive avoidance learning (PA).

Rats were implanted with the cannula aimed at dorsal raphe nucleus. Orexin A or saline were injected into the DRN prior to avoidance training. Pre-training orexin type 1 receptors activation in DRN impaired passive avoidance acquisition but had no effect on PA retention.

Keywords: Dorsal raphe nucleus; Orexin A; passive avoidance learning; rat

1. Introduction

The orexins or hypocretins exist as two neuropeptides named as orexin-A (hypocretin-1) and orexin-B (hypocretin-2), which are derived from the common precursor peptide (preproorexin) (de Lecea et al., 1998; Sakurai et al., 1998). It has been shown that orexin-A has the more potent physiological actions tested in vivo than orexin-B (Edwards et al., 1999; Pu, Jain, Kalra, & Karla, 1998; Sakurai et al., 1998; Shibahara et al., 1999). Orexin A is a 33 amino acid residue with two disulfide bonds whose structure is conserved across many species (Dyer, Touchette, Carroll, Allee, & Matteri, 1999; Sakurai et al., 1998). Orexins act via at least two types of G-protein coupled receptors named the orexin 1 receptor (OX1R) and the orexin 2 receptor (OX2R) (Sakurai et al., 1998).

High levels content of OX1R mRNA was found in the hippocampal formation, tenia tecta, dorsal raphe nucleus (Tao et al., 2006) and most prominently locus coeruleus (LC). While OX2R mRNA was occurred mainly in the cerebral cortex, nucleus accumbens, subthalamic and paraventicular thalamic nuclei (Marcus et al., 2001; Trivedi, Yu, MacNeil, Van der Ploeg, & Guan, 1998).OX2R binds orexin-A and orexin-B with equal affinity, whereas OX1R binds more selectively to orexin-A (Jaeger, 2002; Sakurai, 1998; Trivedi, 1998). Orexin containing neurons in the lateral hypothalamic area produce orexin-A and orexin-B and send their axons to the hippocampus, which predominantly expresses OX1Rs showing a higher affinity to orexin-A (Jaeger, Farr, Banks, & Morley, 2002; Sakurai et al., 1998; Trivedi et al., 1998).

Telegdy and Adamik (2002) have shown that i.c.v. administration of orexin A is able to improve learning, consolidation and retrieval processes in PA paradigm. In recent study the effect of pre-training, post-training and pre-probe trial intrahippocampal CA1 administration of a selective OX1R the orexin 1 receptor antagonist on acquisition, consolidation and retrieval was examined in Morris water maze task (Akbari, Motamedi, Naghdi, & Noorbakhshnia, 2008).Their results have shown that, orexin 1 receptor antagonist impaired acquisition, consolidation and retrieval in spatial learning. This drug had no effect on escape latency of a non-spatial visual discrimination task.

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Therefore, it seems that endogenous orexins, especially orexin-A, in hippocampus play an important role in spatial learning and memory in rat (Akbari et al., 2008). Neuroanatomical studies by using tracing probes have shown that dorsal raphe nucleus (DRN) send efferents via the medial forebrain bundle to hippocampus (Azmitia & Segal, 1978). Orexinergic receptors are distributed in DRN.

As the functional role of orexinergic system in avoidance learning is not clear, the aim of present study was defining the effects of pre-training administration of orexin A into DRN on acquisition and retention in passive avoidance task.

2. Method

2.1. Subjects

Male albino rats 220–250 g, 3-months old obtained from the breeding colony of the Hamadan University of Medical Sciences. Rats were housed five per cage and maintained on a standard 12:12-h light–dark cycle with lights on at 07:00 h. Food and water were available ad lib while the animals adapted to the laboratory and for up to 5 days following surgery.

2.2. Surgery

Approximately 10 days prior to initiation of the behavioral experiments the rats were anesthetized with sodium pentobarbital 50 mg/kg i.p. and were implanted with a cannula-15 mm, 23-gauge aimed at a site immediately above the DRN _AP: -7.8 mm from bregma; ML: 0.0 mm and DV: 4.5 mm below the dura mater according to the atlas of Paxinos and Watson (Paxinos & Watson, 1998). The cannula and two anchoring screws were fixed to the skull with dental cement. Penicillin 0.2 ml i.m. was administered immediately after the surgery.

2.3. Microinjection procedure

Saline and Orexin was injected 10 min before training. Before injection, the animal was restrained by hand; the cannula stylet was removed and replaced with the injection needle30-gauge connected with a short piece of polyethylene tubing to a Hamilton syringe. The needle was inserted 0.5 mm beyond the tip of the cannula. 0.5 µl of saline or orexin (1 µg/ml) was injected during 1 min in saline injected and test groups respectively (Azhdari Zarmehri et al., 2011). The needle was left in place for another 60 s before it was slowly withdrawn. Sham operated group and control group received no injection.

2.4. Step-through passive avoidance (PA) apparatus

This section and following three sections were explained same as our previous paper using this model (Sarihi, 1999). The apparatus used for PA training consisted of a two-compartment box. The larger illuminated chamber 35×20×15 cm^3 made from transparent plastic was connected by an 8x8 cm^2 guillotine door to the smaller dark compartment 25×15×15 cm^3 with black opaque walls and ceiling. The floor of the dark compartment was constructed of stainless steel rods 3 mm in diameter, 10 mm apart. Through which foot-shock could be delivered from a constant current source.

2.5. Training procedure

All experimental groups were first habituated to the apparatus. The rat was placed in the illuminated compartment and 30 s later the guillotine door was raised. Upon entering the dark compartment the door was closed and the rat was taken from the dark compartment into the home cage. The habituation trial was repeated after 30 min and followed after the same interval by the acquisition trial during which the guillotine door was closed and a 50 Hz, 0.8 mA constant current shocks was applied for 2 s immediately after the animal had entered the dark compartment. After 20 s, the rat was removed from the dark compartment and placed into the home cage. The rat was retained in the apparatus and received a foot-shock each time if re-entered the dark compartment. Training was terminated when the rat remained in the light compartment for 120 consecutive seconds. The number of trials entries into the dark chamber was recorded.

2.6. Retention test

The retention test was performed 48 h after the IA acquisition training. Similar to acquisition training, the rat was placed in the illuminated compartment facing away from the door. After 5 s, the guillotine door was raised and the rat was allowed to enter the dark compartment. To assess memory retention, the step-through latency and the time spent in the dark compartment (TDC) were recorded for up to 600 s. If the rat did not enter the dark compartment within 600 s, the retention test was terminated and a ceiling score of 600 s was assigned.

2.7. Histology

At the end of each experiment, the animals were deeply anesthetized with sodium pentobarbital and 1 µl pontamine sky blue was injected via the injection cannula. The perfusion–fixation was performed intracardially with saline followed by 10% formalin phosphate buffer solution. The brains were then removed and post-fixed in the same fixative. Then the paraffin-sections 20 m thick were stained with H and E staining for histological examination. The location of cannula was verified by examining enlarged projections of the slides. The volume of drug injected into the DRN in this experiment has been reported to spread from 0.5 to 1.5 mm from the site of injection (Sarihi, Motamedi, Rashidy-Pour, Naghdi, & Behzadi, 1999). Therefore, a cannula positioned more than 0.5 mm from the intended site of injection was not considered in statistical analysis. We used 37 rats in four

experimental groups. In three operated animals, cannula tips deviated by more than 0.5 mm from the target structure and were excluded from statistical analysis. In the remaining animals, all DRN cannula tips were just above the DRN and were considered to have correct placements Fig. 1 representative illustrating the location of cannula aimed at the DRN

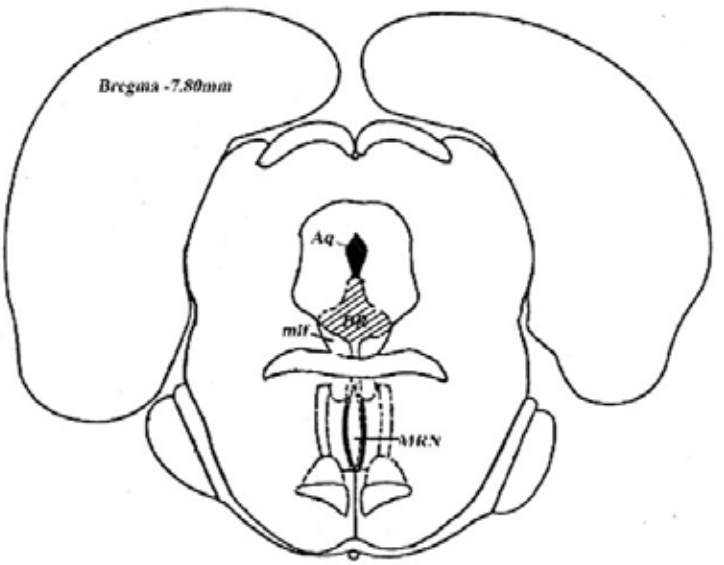

Fig. 1. A coronal plans through the injection site (asterisks) in dorsal raphe nucleus. Aq, aqueduct; mlf, medial longitudinal fasciculus; MRN, median raphe nucleus; DR, dorsal raphe nucleus. Adopted from (Paxinos & Watson, 1998)

2. 8. Statistical analysis

Statistical evaluation was done by Kolmogorov-Smirnov test at first, to test normal distribution. Evaluation of significant differences of number of trials was done by Mann-Whitney U-test. Step-through latency and time spent in dark compartment was compared using one-way *ANOVA* followed by Tukey's test for multiple comparisons. All results have shown as mean $\pm$ *SEM*. The level of $p < 0.05$ was considered significant.

3. Results

3. 1. Effect of pre-training orexin injection into the DRN on PA acquisition

Comparison of the number of trials to acquisition shows there is significant difference between control (1.62 $\pm$ 0.32) and orexin injected (3.5 $\pm$ 0.52) groups (Fig. 2). There were no significant differences among control, sham operated and saline injected groups in number of trials. Results indicates that ORX1 receptors activation in DRN impair passive avoidance acquisition.

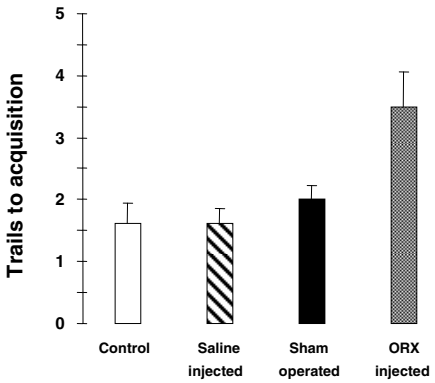

Fig. 2. The effects of pre-training orexin injection into the DRN on PA learning. Ordinate: mean ($\pm$ *SEM*) trials to acquisition

3. 2. Effect of pre-training orexin injection into the DRN on PA retention

The results of retention test 48 hour after training is summarized in Fig. 3 (A, B). *ANOVA* analysis showed absence of significant difference between the step-through latency, time spent in dark compartment and number of entrance of two groups, indicating that pre-training orexin injection into the DRN has no effect on PA retention.

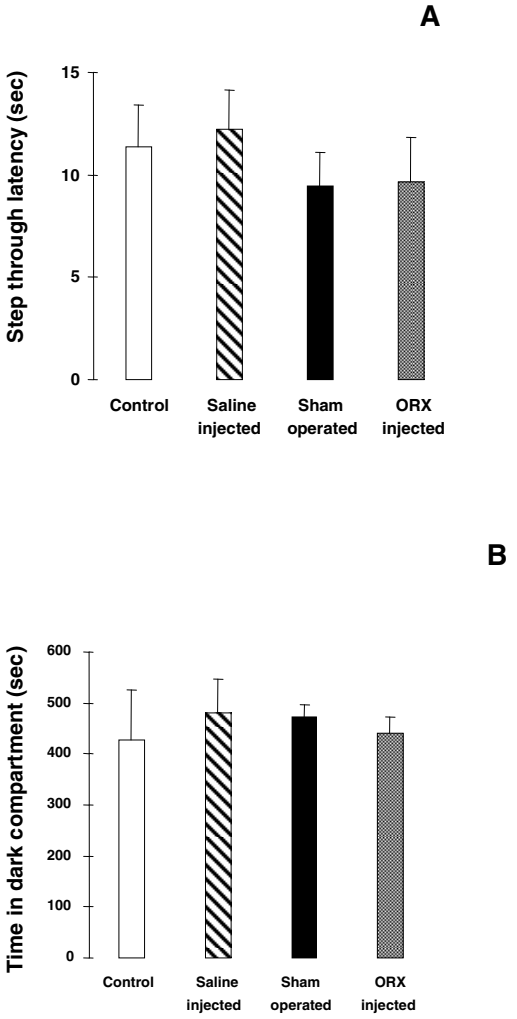

Fig. 3. The effect of pre-trainng orexin injection into the DRN on PA retention. Ordinate: mean (± *SEM*) step-through latency (A) and time spent in dark compartment (B) during the retrieval test performed 2 days after PA acquisition

4. Discussion

Our results indicated that:

1. ORX1 receptors activation in DRN impairs passive avoidance acquisition.
2. Pre-training orexin injection into the DRN on has no effect on PA retention.

Neuroanatomists have shown that DRN send efferents via the medial forebrain bundle to hippocampus (Azmitia & Segal, 1978). High levels content of OX1R mRNA was found in the hippocampal formation, tenia tecta, and dorsal raphe nucleus (Tao et al., 2006). Based on the literature the role of activation or blocked of orexinergic receptors in dorsal raphe and neuronal pathways which are responsible in cognitive responses of the orexinergic system into the dorsal raphe remains to be examined.

Telegdy and Adamik have shown that i.c.v. administration of orexin A is able to improve learning in PA paradigm (2002). Our founding is in contrast with their report, which may be due to that in our study we injected the orexin locally into the dorsal raphe but in Telegdy and Adamiks' experiment orexin may act through different parts of the brain. It has been shown that orexin receptor blockade can impair acquisition (Akbari et al., 2008). Thus, the observed effect by Telegdy and Adamik is mainly due to hippocampal orexin receptor activation, but the orexinergic system in DRN may exert different role in acquisition of PA.

As we mentioned in introduction section, Akbari et al., (2008) have shown that, orexin 1 receptor antagonist impaired acquisition of spatial learning in Morris water maze task. We showed orexin activation in dorsal raphe impair passive avoidance learning. The discrepancy between our data and their results may be due to the difference in behavioral model of learning and memory which different neuronal circuits may involve in each model. Therefore, orexinergic system may play different role in spatial learning and avoidance learning. On the other hand not only the site of orexinergic system but also blocking or activation of this system through different neuronal pathways or different mechanisms may act on memory processing which remain to be elucidated.

In this study we inject orexin 10 min before training, while Ehrström et al. (2004) reported orexin half time and based on their report orexin may affect until 27 minutes. We know that consolidation of memory start immediately after acquisition and take several hours or even longer. Therefore in our result orexin in DRN may have affected passive avoidance consolidation stage. In other words the role of orexinergic system on different stage of avoidance learning such as consolidation remains to be tested.

In conclusion, pretraining DRN orexinergic system activation impairs conditioning learning such as passive avoidance, and has no effect on retrieval stage. However, the role of this system on the consolidation and its activity before memory storage has to be study.

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