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Intrahippocampal Injections of Thyrotropin-Releasing Hormone(TRH) Facilitate Trace Conditioning of the Rabbit's Eyeblink Response.

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Abstract

Thyrotropin-releasing hormone(TRH) and its receptors are widely distributed throughout the hippocampus. It plays some role in neurotransmission and neuromodulation and may affect on learning and memory functions. In the present study the effects of intrahippocampal microinjections of TRH(1,3 and 10?g/?l) on the trace classical conditioning of the rabbit's eyeblink were examined. Five minutes before starting any training sessions, 1 ?l of TRH solution was injected through an implanted cannula into the CA1 area of hippocampus. The results indicate that TRH, dose dependently, increased the percentage of conditioned responses and shortened the latency of responses, compared with those in the control group. Therefore, it seems that TRH facilitates eyeblink trace conditioning and it may have an improving action on learning and memory retention.

Keywords: *Classical conditioning-thyrotropin releasing hormone (TRH)-hippocampus-learning-trace conditioning-memory*

Introduction

In addition to its hypophyseotropic effects, thyrotropin-releasing hormone(TRH) has several other actions (neuroprotective, autonomic, and anticonvulsant) in the central nervous system (Faden *et al.*, 1999; Knoblach & Kubek, 1997; Pizzi *et al.*, 1999). TRH and its receptors are widely distributed throughout the brain (Eymin *et al.*,1993; Manaker *et al.*, 1986).This tripeptide may play some role in neurotransmission and neuromodulation and has stimulatory effect on

acetylcholine and catecholamines release from frontal cortex and hippocampus (Giovanini *et al.,* 1991; Okada, 1991; Toide *et al.*, 1993).There are considerable evidences that septo-hippocampal cholinergic pathway has important role in learning and cognitive functions (Emerich *et al.,* 1992; Galey *et al.,* 1994; Maeda *et al.*, 1994). It has been reported that systemic administration of TRH or its analogs improve significantly some symptoms in Alzheimer's disease (Mellow *et al.,* 1989). Some investigations have shown that these peptides have learning and memory improving actions in rat (Shinoda *et al.,* 1999; Yamamoto *et al.,* 1990; Yamamura *et al.*, 1991).Therefore, it is suggested that intrahippocampal TRH may ameliorate learning and memory retention in various tasks through facilitation of cholinergic pathway.

In order to investigate the central effect of lower doses of TRH on learning and memory, we examined the effect of intrahippocampal microinjections of TRH on trace classical conditioning of rabbit's eyeblink response. Since the hippocampus has been proved to be more involved in trace than usual delay paradigm, we selected this brain structure as the target organ for drug injections (Sprick, 1995; Woodruff-Pak *et al.,* 1990).

The eyeblink classical conditioning as an animal model has some advantages for evaluating the effects of experimental manipulations on learning and memory (Solomon & Pendlebury, 1994). The behavior and neurobiology of this simple form of learning are well understood and there are similarities between human and rabbit (Solomon & Pendlebury, 1994; Woodruff-Pak *et al.,* 1990). In the present study, we used young male rabbits, because the age and sex hormones affect learning behaviors (Haaren *et al.,* 1990; Yamamoto *et al.,* 1990).

Materials and methods

Subjects: The animals were 27 young adult male albino rabbits (*Oryctolugus cuniculus*) weighing 1.9±0.3kg and 4-6 months of age (Inbred in Animal House of Shiraz Medical School). Rabbits were taken to the laboratory two weeks before the surgical operation. They were individually housed in standard rabbit cages; Food and water were continuously available.

Surgical procedures: One week before the beginning of behavioral training, rabbits were anesthetized with intravenous injection of sodium pentobarbital (40 mg/kg body weight) and a loop of 5-0 silk thread was sutured into the middle of upper eyelid. A guide cannula was implanted into the CA1 area of the dorsal hippocampus using stereotaxic coordinates (4.0 mm posterior, 4.0 mm lateral and 5.8 mm ventral to Bregma) according to Atlas of Girgis (Girgis & Shin, 1981). The cannula and a transducer base were cemented with anchor screws on the skull. Animals were given one week for recovery and then behavioral training began.

Training procedures: All animals were trained using trace classical conditioning paradigm described by Woodruff-Pak and coworkers (Woodruff-Pak, 1993). One day prior to training, each animal was adapted to the restraint of the apparatus for 60 minutes. Animals were placed in the training box where they received sound and air puff stimuli. The conditioned stimulus (CS) was a 250 ms tone (1000 Hz & 85 dB) and the unconditioned stimulus (US) was a 100 ms air puff (200 gr/cm2) applied to the cornea. The period between CS and US onset was 750 ms; there was a trace interval (dead period) of 500 ms (Fig.1).

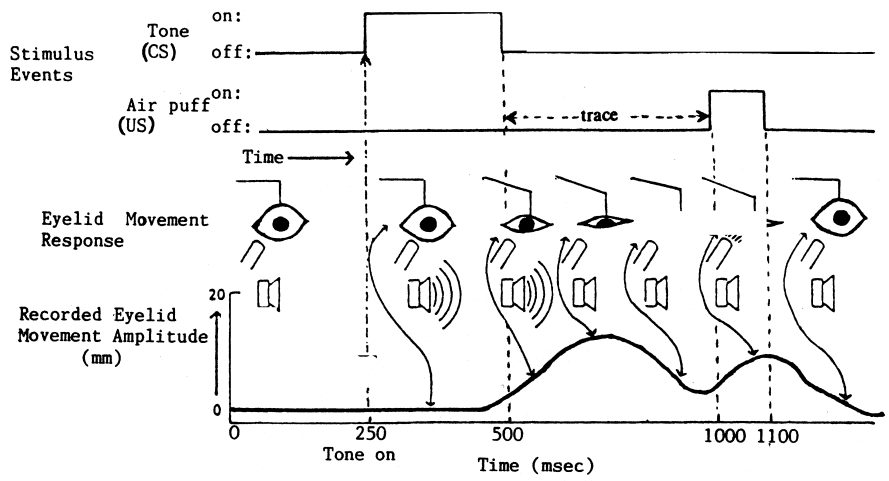

Figure 1 - Demonstration of a training trial events and periods that CS and US are applied in trace paradigm of eyeblink conditioning. Lower trace showes a recorded conditioned response of eyelid movement.

The rabbits were trained daily with one session consisting 12 blocks of 9 trials. The inter-trial interval periods in all sessions were 30 sec. Dividing session to blocks was only for ease of statistical analysis. Eyelid movement was measured by a minitorque potentiometer attached to the eyelid through the sutured loop. The CS and US timing, presentation and recording of eyelid response were performed by a computer equipped with an appropriate A/D board (8 byte, 8 output channel with A/D system and 2 timer).

Training was continued until the animal reached a criterion of 80% or more conditioned responses in any session and it was discontinued after 12 training sessions, if the behavioral criterion had not been reached. The memory test was done 24 hr after the last training session(Criterion session) with no presentation of US.

Drug administration: Before starting each session, freshly prepared solutions of 1?à/?À TRH (n=6), 3?à/?À TRH (n=8), 10 ?à/?À TRH (n=6) or vehicle (1?l of artificial cerebro-spinal fluid "ACSF" in control group, n=7) was injected, with a 30 gauge needle attached to a PE-10 tube by a Hamilton syringe, into the dorsal hippocampus (CA1) by using microinjection pump (over a 5-min period, at an infusion rate of 0.2 ?l/min).

At the end of training, and after memory test, the animals were sacrified with an overdose of pentobarbital and perfused with saline followed by 10% formalin through the carotid arteries. Brains were maintained in formalin for 4 or more days. Frozen sections were taken at 80 ?¦m and stained with cresyl violet and examined under the microscope, checking the position of cannula tip.

Data analysis

All acquisition data of each block or session including the percentage of conditioned responses (%CRs) and latency of eyeblink responses are reported in Mean±SE. Data were statistically analysed by one way ANOVA and t-test. Differences were considered significant if P value calculated from student's t-test was smaller than 0.05.

Results

The dose-response effects of TRH on learning and memory retention are shown in Fig.2 and 3 respectively. As revealed from Figure 2 , TRH at 3 and 10 ?8/ul significantly increased the percentage of conditioned responses (%CRs) after the third session (P<0.01).

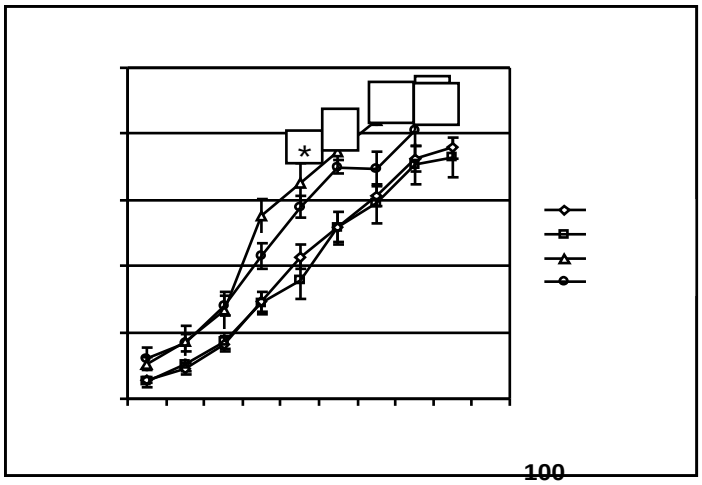

Figure 2 - Effects of different doses of TRH on acquisition of eyeblink conditioning in rabbits. The Mean+SE percentage of conditioned responses (%CRs) of the training sessions (days) are compared in different groups (* =P<0.05).

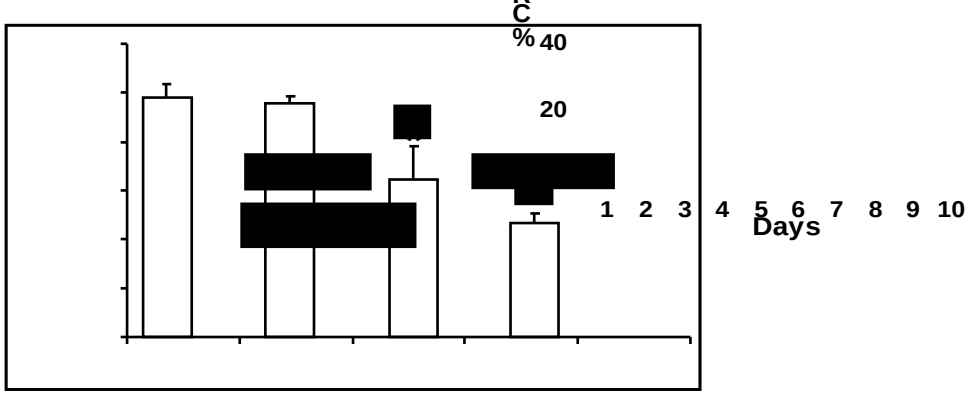

Figure 3 - Effects of pre-training injections of different doses of TRH (1,3,10ug/ul) on latency of conditioned responses (Mean+SE) during memory test session compared to those of the control (CTRL) group (* = P<0.05).

760
720
680

The latency of CRs during memory test, in groups receiving TRH (3 or 10?â) were shorter than that in the control group (P<0.01). As shown in Fig. 4 CR number of memory blocks in group receiving TRH (3?g) were significantly more than that in control group (P<0.01). After the sixth block, CR number usually reached to zero (Extinction of responses). The rabbits receiving high dose of TRH (10 ?g) often showed behavioral excitation like sniffing, increase in respiratory rate and marked reactivity, few minutes after drug injection. Therefore, in the next studies we used 3?É/?Éof TRH. The mean number of session (Days) required for reaching the learning criterion in TRH receiving and control groups were 6.68±1.22 and 8.88±0.61 respectively. The difference between TRH-treated and control group is statistically significant (P<0.05). The mean of total number of CRs of the first 6 sessions in TRH-treated rabbits (185.1±21.5) were markedly (P<0.005) more than that in control group (111.8±17.3).

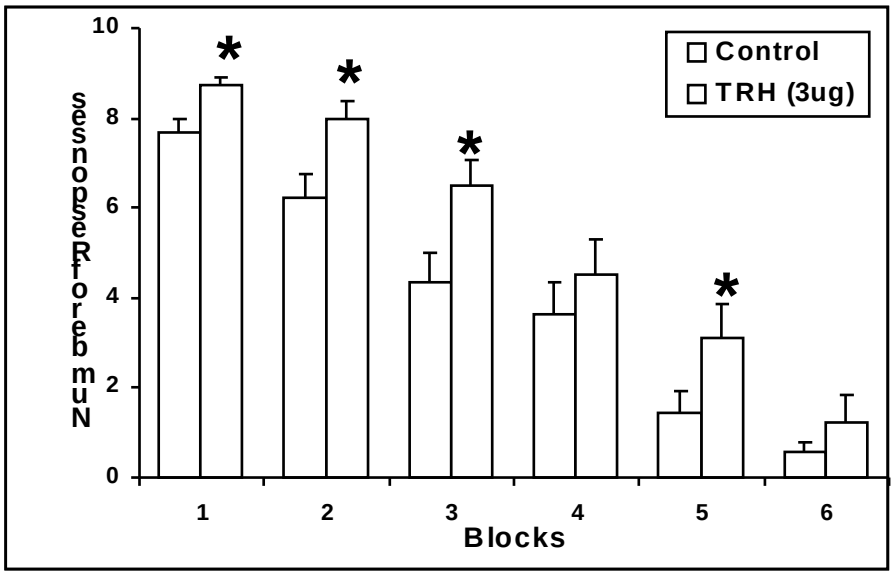

Figure 4 - Effect of TRH (3ug/ul) on the number of conditioned responses (Mean+SE) of the first 6 blocks of memory test session (* P<0.01).

Discussion

One of the major discovery of the past two decades has been that, neuropeptides play a significant role in the processes involved in learning and memory (Kovacs & De Wied, 1994; Bennett *et al.*, 1997). Most of the neuropeptides, which are widely distributed in the brain affect neuronal excitability and modify behavior (Bennett *et al.*, 1997). The present study demonstrated that intrahippocampal microinjections of TRH, dose dependently has facilitating action on the eyeblink conditioning, in rabbits. The results support new evidences that TRH and its analogs have a potent learning and memory improving action in rodents and in Alzheimer's disease (Mellow *et al.*, 1989; Shinoda *et al.*, 1999). There are a number of reports indicating that systemic administration of TRH or its analogs improve the memory impairments in various tasks (Faden *et al.*, 1999; Miyamoto *et al.*, 1993; Ogasawara *et al.*, 1996). Since the hippocampus has modulatory action on learning and memory retention, and it is much involved in trace conditioning, the local microinjection of lower doses of TRH into the dorsal hippocampus had an effective improving action on memory (Sprick, 1995; Woodruff-Pak *et al.*, 1990).

TRH and its analogs have been shown to stimulate the cholinergic, noradrenergic and dopaminergic systems by enhancing release of the neurotransmitters (Ogasawara *et al.*, 1996; Toide *et al.*, 1993). Since septo-hippocampal cholinergic pathway has important role in learning and cognitive functions, TRH action may be through enhancing acetylcholine release (Galey *et al.*, 1994; Giovanini *et al.*, 1991; Lamour *et al.*, 1989). The effect of higher dose of TRH on stereotype behavior indicates that it may interact with dopaminergic mechanisms (Ogasawara *et al.*, 1996) Therefore, the lesser facilitating effect of high dose of TRH (10 ?g) on acquisition and memory in our experiments may be related to its behavioral excitation effect. These observations suggest that TRH in hippocampus may act as a neuromodulator and/or neurotransmitter and it has some role in learning and memory consolidation. Although it is far too early to determine its exact role, there exists some evidence that TRH modulates learning and memory processes at the behavioral, the cellular, and the synaptic level (Kovacs & De Wied, 1994). However, further studies are required to elucidate

the precise mechanisms of TRH interactions with other neurotransmitters of hippocampus involved in learning and memory functions.

Considering that eyeblink conditioning is a standard type of learning and its neural mechanisms between human and rabbit are similar, makes it a potentially useful preparation, to evaluate pharmacological agents that facilitate learning and memory, and to study disorderes of learning and memory (Solomon *et al.*, 1993; Solomon & Pendlebury, 1994). Therefore, it may be possible to extrapolate the results from rabbits to humans. For instance, it has been reported that in patients with Alzheimer's disease (age related memory disorderes) eyeblink conditioning response is also disrupted (Solomon *et al.*, 1991).

As a result, it seems that intrahippocampal injections of TRH facilitate trace classical condioning of the rabbit's eyeblink response and endogenous TRH may have neuromodulatory role in learning and memory functions.

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