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ScienceDirect
Neurobiology of Learning and Memory 89 (2008) 370–378
Neurobiology of Learning and Memory
www.elsevier.com/locate/ynlme

Post-training administration of corticotropin-releasing hormone (CRH) enhances retention of a spatial memory through a noradrenergic mechanism in male rats

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Received 9 December 2005; revised 17 October 2007; accepted 17 October 2007
Available online 20 December 2007

Abstract

Hormones released in response to stress play important roles in cognition. In the present study, the effects of the stress peptide, corticotropin-releasing hormone (CRH), on spatial reference memory were assessed following post-training administration. Adult Long-Evans male rats were trained for 6 days on a standard water maze task of reference memory in which animals must learn and remember the fixed location of a hidden, submerged platform. Each day, immediately following three training trials, rats received bilateral infusions of CRH into the lateral ventricles over a range of doses (0.1, 0.33, 1.0, 3.3 μg) or a vehicle solution. Post-training infusions of CRH improved retention as indicated by significantly shorter latencies and path lengths to locate the hidden platform on the first training (retention) trial of days 2 and 3. Additionally, post-training administration of CRH increased spatial bias during probe trials as measured by proximity to the platform location. CRH did not enhance performance on retention or probe trials when administered 2 h after daily training indicating that CRH facilitated consolidation specifically. The effects of CRH were attenuated by intraventricular co-administration of the beta-adrenergic antagonist, propanolol, at bilateral doses that had no effect on retention alone (0.1, 1.0 μg). Results indicate that post-training administration of CRH enhanced spatial memory as measured in a water maze, and this effect was mediated, at least partly, by a noradrenergic mechanism.

Keywords: Corticotropin-releasing hormone; Propranolol; Water maze; Memory; Noradrenergic

1. Introduction

Corticotropin-releasing hormone (CRH), the 41 amino acid peptide that initiates the neuroendocrine stress cascade, has been implicated as a neuromodulator of learning and memory in rodents (Behan et al., 1995; Eckart et al., 1999; Heinrichs & Koob, 2004; Heinrichs et al., 1997; Koob & Bloom, 1985; Lee, Lee, Wang, & Lin, 1993; Lee & Sung, 1989; Thompson, Erickson, Schulkin, & Rosen, 2004). CRH and its cognate receptors and associated mRNA are distributed broadly throughout regions of the nervous system implicated in mnemonic processes (Bittencourt & Sawchenko, 2000; De Souza & Battaglia, 1988; Sawchenko et al., 1993; Swanson, Sawchenko, Rivier, & Vale, 1983; Van Pett et al., 2000). Intracerebroventricular administration of CRH and related peptides facilitated acquisition and enhanced retention on a number of tasks assessing learning and memory in rodents, including inhibitory avoidance, visual discrimination, social learning, and spatial mapping (Behan et al., 1995; Chen, Chiu, & Lee, 1992; De Souza, 1995; Heinrichs, 2003; Hung, Chou, Chiu, & Lee, 1992; Liang, Chen, & Chen, 2001; Liang & Lee, 1988; Zorrilla et al., 2002).

Intraventricular administration of CRH to male rats prior to training trials was found to enhance learning during acquisition of a water maze (Zorrilla et al., 2002). However, central administration of CRH induces a

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doi:10.1016/j.nlm.2007.10.008

constellation of behavioral responses indicating an increased level of arousal of the organism, possibly related to its endocrine and autonomic actions (Heinrichs, Menzaghi, Merlo Pich, Britton, & Koob, 1995; Koob & Thatcher-Britton, 1985). These behavioral effects of CRH administration can complicate the study of CRH modulation of learning and memory because performance can be affected by the sensory, motivational, and/or motor effects of CRH without directly altering the neurobiological systems underlying cognition (McGaugh, 1983). Post-training administration is a procedure typically used to dissociate the effects of exogenous agents on non-mnemonic factors from their effects on the brain systems that mediate learning and memory (Gold & van Buskirk, 1975; McGaugh, 1983). In this paradigm, compounds under study are administered immediately following training sessions and their effects on retention usually are tested the following day well after the drug or hormone has cleared the system. Theoretically, agents administered on this schedule affect consolidation of information learned during training without altering non-mnemonic processes (McGaugh, 2000). This conclusion is reinforced by demonstrations that treatments given beyond 2 h after training usually are ineffective in influencing retention the following day.

The post-training paradigm was used previously in rats to demonstrate that administration of CRH into the bed nucleus of the stria terminalis or lateral ventricles immediately following one-trial training enhanced retention on an inhibitory avoidance task (Liang et al., 2001; Zorrilla et al., 2002) while administration of a CRH antagonist into the amygdala impaired retention on this task (Roozendaal, Brunson, Holloway, McGaugh, & Baram, 2002). Similarly, post-training administration of CRH into the hippocampus of mice enhanced a conditioned fear response (Radulovic, Ruhmann, Liepold, & Spiess, 1999) and post-training infusion of a CRH antagonist into the amygdala impaired memory of a social defeat (Robison et al., 2004). We hypothesized that CRH administered immediately after training on a standard water maze task would similarly enhance retention for the platform location on the next day confirming that CRH has broad effects on memory consolidation that extend to strongly spatial forms of learning and memory.

CRH and CRH-related peptides are capable of directly affecting neurotransmitter systems implicated in learning and memory, including noradrenergic, cholinergic, and glutamatergic systems (Lee et al., 1993; Onali & Olianas, 1998; Tizabi & Calogero, 1992). At the cellular level, CRH may influence signaling processes involved in the consolidation of memory through an interaction with NMDA receptor-dependent mechanisms, either directly via its own receptor, or by altering noradrenegic and cholinergic systems (Lee et al., 1993; Sauvage & Steckler, 2001; Steckler & Holsboer, 2001). Given that central noradrenergic systems have been implicated in the effects of CRH on learning and memory (Chen et al., 1992; Lee et al., 1993), we also tested the hypothesis that the effects of post-training administration of CRH on retention of spatial information could be blocked by the beta-noradrenergic antagonist propanolol.

2. Materials and methods

2.1. Experiment 1: Effects of post-training CRH administration on retention in a water maze

2.1.1. Subjects

Experiments were performed on adult Long-Evans male rats (300–400 g) obtained from Charles River, Inc. (Wilmington, MA). The rats were housed individually in a temperature-controlled vivarium maintained at 21 °C on a 12:12 h light/dark cycle with lights on at 7:00 am. Food (Purina Rat Chow, Purina Mills, St. Louis, MO) and water were available *ad libitum*. All experimental procedures were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1996) and were approved by the Tulane University Institutional Animal Care and Use Committee.

2.1.2. Stereotaxic cannulation

Rats were anesthetized by intraperitoneal injection with ketamine (100 mg/kg, Bristol Laboratories, Syracuse, NY) and xylazine (7 mg/kg, Miles Laboratories, Shawnee, KS), and placed into a stereotaxic frame for bilateral implantation of indwelling cannulae into the lateral ventricles. Surgeries were conducted under aseptic procedures. Bilateral guide cannulae constructed of 23-ga stainless-steel tubing (Small Parts, Inc., Miami Lakes, FL) were lowered through trephine holes to 1 mm above the target site in the lateral ventricles and anchored to the skull with dental acrylic attached to three stainless-steel machine screws (Small Parts, Inc.). The guide cannulae were placed bilaterally at coordinates 0.26 mm posterior to bregma, 1.5 mm lateral to the mid-line, and 3.1 mm ventral to the skull surface (Paxinos & Watson, 1998). Inner cannulae constructed of 28-ga stainless-steel tubing (Small Parts, Inc.) extended 1 mm beyond the tips of the guide cannulae into the lateral ventricles to maintain patency between infusions. Following surgery, all rats received systemic injections of an analgesic to reduce pain (butorphanol tartrate, 2 mg/kg, Bristol Laboratories, Syracuse, NY) and recovered fully without complications. The animals were allowed to recover for 1 week before the first day of training and treatment.

2.1.3. Behavioral testing and drug infusions

To assess the effects of CRH on spatial learning and memory, subjects were tested under a distributed training schedule in a standard rat water maze. The water maze consisted of a galvanized circular pool, 180 cm in diameter and 50 cm in height, painted with a non-toxic white epoxy (Insl-X Product Co., Yonkers, NY). The pool was filled with tap water to a level of 39 cm made opaque by the addition of 1500 ml of white tempera paint (Sargent Art, Inc., Hazelton, PA) and maintained at a temperature of 25 °C during testing. Fixed extramaze cues were attached to a black curtain surrounding the water maze. The time (latency) and distance (path length) required to escape and proximity to a Plexiglas platform, 12 cm in diameter and submerged 2 cm below the water surface, were recorded by a video camera and recorder interfaced with a behavioral tracking system (HVS Imaging, Hampton, UK).

Rats received one training session per day consisting of three trials for six consecutive days. On each trial the rat was placed into the pool facing the wall at one of the four designated start points (N, S, E, or W) and allowed to escape onto the hidden platform, which remained in the same pool location throughout the entire experiment. If the rat failed to escape within 60 s, it was manually guided to the platform. The rat was allowed to remain on the platform for 15 s before being placed in an opaque holding cage for 30 s between trials. A different starting point was used on each trial on a pseudorandom schedule so that no starting point was used twice during a daily training session. Additionally, after every sixth training trial (i.e., every other day), a probe trial, in which the platform was removed in