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Memory-enhancing corticosterone treatment increases amygdala norepinephrine and Arc protein expression in hippocampal synaptic fractions

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ABSTRACT

Considerable evidence indicates that glucocorticoid hormones enhance the consolidation of memory for emotionally arousing events through interactions with the noradrenergic system of the basolateral complex of the amygdala (BLA). We previously reported that intra-BLA administration of a β-adrenoceptor agonist immediately after inhibitory avoidance training enhanced memory consolidation and increased hippocampal expression of the protein product of the immediate early gene activity-regulated cytoskeletal-associated protein (Arc). In the present experiments corticosterone (3 mg/kg, i.p.) was administered to male Sprague-Dawley rats immediately after inhibitory avoidance training to examine effects on long-term memory, amygdala norepinephrine levels, and hippocampal Arc expression. Corticosterone increased amygdala norepinephrine levels 15 min after inhibitory avoidance training, as assessed by *in vivo* microdialysis, and enhanced memory tested at 48 h. Corticosterone treatment also increased expression of Arc protein in hippocampal synaptic tissue. The elevation in BLA norepinephrine appears to participate in corticosterone-influenced modulation of hippocampal Arc expression as intra-BLA blockade of β-adrenoceptors with propranolol (0.5 μg/0.2 μL) attenuated the corticosterone-induced synaptic Arc expression in the hippocampus. These findings indicate that noradrenergic activity at BLA β-adrenoceptors is involved in corticosterone-induced enhancement of memory consolidation and expression of the synaptic-plasticity-related protein Arc in the hippocampus.

1. Introduction

Events associated with emotional arousal are typically well remembered. Such memory enhancement involves the release of the adrenal stress hormones epinephrine and glucocorticoids (Cahill & Alkire, 2003; McGaugh, 2004; Roozendaal, McEwen, & Chattarji, 2009; Roozendaal, Quirarte, & McGaugh, 1997; Van Stegeren et al., 2007). Systemic administration of either epinephrine or the glucocorticoid corticosterone enhances memory consolidation when given immediately after training on a variety of emotionally arousing learning tasks, including inhibitory avoidance, spatial water maze, conditioned taste aversion and object recognition training (Gold & van Buskirk, 1975; Miranda, Quirarte, Rodriguez-Garcia, McGaugh, & Roozendaal, 2008; Okuda, Roozendaal, & McGaugh, 2004; Roozendaal, Hahn, Nathan, de Quervain, & McGaugh, 2004; Roozendaal, Portillo-Marquez, & McGaugh, 1996). Extensive evidence that blockade of β-adrenoceptors in the basolateral complex of the amygdala (BLA) prevents the memory-enhancing effects of peripherally administered stress hormones, indicates that noradrenergic activity in the BLA is critically involved in enabling stress hormone effects on memory consolidation (Liang, Juler, & McGaugh, 1986; Quirarte, Roozendaal, & McGaugh, 1997; Roozendaal et al., 2009). There is also considerable evidence that post-training infusions of norepinephrine or β-adrenoceptor agonists into the BLA enhance memory consolidation (Ferry & McGaugh, 1999; Gallagher, Kapp, Musty, & Driscoll, 1977; Hatfield & McGaugh, 1999; LaLumiere, Buen, & McGaugh, 2003; McIntyre et al., 2005; Miranda, LaLumiere, Buen, Bermudez-Rattoni, & McGaugh, 2003; Roozendaal, Castello, Vedana, Barsegyan, & McGaugh, 2008). Moreover, inhibitory avoidance training increases norepinephrine levels in the BLA and the increase is positively correlated with subsequent memory of the training (McIntyre, Hatfield, & McGaugh, 2002). Systemically administered epinephrine also increases norepinephrine levels in the brain (Gold & van Buskirk, 1978) including the amygdala (Williams, Men, Clayton, & Gold, 1998). Although prior studies

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reported that glucocorticoids interact with the noradrenergic system of the BLA to influence memory consolidation, it is not known whether corticosterone influences norepinephrine levels in the BLA.

Stress hormone and training-induced amygdala activation modulates synaptic plasticity in brain areas engaged at the time of memory consolidation (McGaugh, McIntyre, & Power, 2002). Noradrenergic activity in the BLA appears to be necessary for mediating stress effects on other brain regions involved in memory, as blockade of β-adrenoceptors in the BLA prevents inhibitory avoidance memory enhancement induced by a glucocorticoid receptor (GR) agonist administered directly into the hippocampus (Roozendaal, Nguyen, Power, & McGaugh, 1999). Similarly, both GR and β-adrenoceptor activation in the BLA influence hippocampal dentate gyrus long-term potentiation (LTP) (Akirav & Richter-Levin, 2002; Ikegaya, Nakanishi, Saito, & Abe, 1997; Vouimba, Yaniv, & Richter-Levin, 2007). We previously reported that stimulation of β-adrenoceptors within the BLA after inhibitory avoidance training enhances memory and increases the expression of activity-regulated cytoskeletal-associated (Arc) protein levels in the dorsal hippocampus without influencing *Arc* mRNA levels (McIntyre et al., 2005). This finding suggests that amygdala modulation of Arc protein and synaptic plasticity in the hippocampus occurs at a post-transcriptional level.

Hippocampal expression of Arc is known to play a role in the maintenance of long-term plasticity and memory. Inhibition of Arc protein expression with infusions of antisense oligodeoxynucleotides (AS ODNs) into the dorsal hippocampus impairs the maintenance of LTP without affecting its induction (Guzowski et al., 2000; Messaoudi et al., 2007). Similarly, intra-hippocampal infusions of AS ODNs impair long-term, but not short-term memory for spatial water-maze and inhibitory avoidance tasks (Guzowski et al., 2000; McIntyre et al., 2005). These findings are consistent with evidence of impaired long-term memory and abnormal synaptic plasticity in transgenic mice lacking the *Arc* gene (Plath et al., 2006). The evidence that *Arc* is translated in synapses *in vitro* (Bloomer, VanDongen, & VanDongen, 2007; Sanders, Happe, Bylund, & Murrin, 2008; Waung, Pfeiffer, Nosyreva, Ronesi, & Huber, 2008; Yin, Edelman, & Vanderklish, 2002), suggests that the influence of emotional arousal and stress hormones on memory consolidation involves an amygdala-mediated influence on local translation of synaptic proteins, such as Arc, in hippocampal synapses.

Here, we examined glucocorticoid effects on amygdala norepinephrine levels and hippocampal Arc expression in regulating the consolidation of memory of inhibitory avoidance training. To investigate the effect of corticosterone on amygdala norepinephrine levels, rats were given systemic injections of a memory-enhancing dose of corticosterone immediately after training and norepinephrine levels in the amygdala were measured with *in vivo* microdialysis and high-performance liquid chromatography (HPLC). Western blot analysis and immunohistochemistry were used to determine whether memory-enhancing corticosterone treatment affects the expression of hippocampal Arc protein. The role of amygdala norepinephrine in mediating corticosterone-induced changes in hippocampal Arc expression was examined by blocking β-adrenoceptors with infusions of propranolol into the BLA immediately following post-training corticosterone treatment.

2. Materials and methods

2.1. Subjects

Two-hundred-and-seventy-nine male Sprague-Dawley rats (250–275 g upon arrival), obtained from Charles River Breeding Laboratories (Wilmington, MA), were housed individually in a temperature-controlled (22 °C) colony room, with food and water available *ad libitum*. Animals were maintained on a 12 h light–12 h dark cycle (7:00–19:00 h, lights on) and kept in the animal colony for 1 week before commencement of surgical or behavioral procedures. All experimental procedures were in compliance with the National Institutes of Health guidelines and were approved by the Institutional Animal Care and Use Committee (University of California, Irvine and University of Texas at Dallas).

2.2. Surgery

Rats used in the microdialysis experiment were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), given atropine sulfate (0.4 mg/kg, i.p.) to maintain respiration, and a unilateral plastic guide cannula (CMA 12, Carnegie Medicin, North Chelmsford, MA, USA) for a microdialysis probe was implanted 1 mm above the left BLA [coordinates: anteroposterior (AP), −2.8 mm from Bregma; mediolateral (ML), +4.8 mm from midline; dorsoventral (DV), −6.6 mm below skull surface; incisor bar, −3.3 mm from interaural line (Paxinos & Watson, 2005)]. Animals for the intra-BLA infusion experiment were anesthetized with isoflurane (1% in O_2) (Western Medical Supply) and given atropine sulfate. The skull was positioned in a stereotaxic frame (Stoelting, Wood Dale, Il) and two 15-mm-long guide cannulae (23 gauge; Small Parts, Miramar, Fl) were implanted bilaterally with the tips 2 mm above the BLA (AP, −2.7 mm; ML, ±5.2 mm; DV, −6.4 mm). The guide cannulae were fixed in place with acrylic dental cement and two small anchoring screws. Stylets (15-mm long insect dissection pins) were inserted into each cannula to maintain patency. After surgery, rats were given 2.0 mL of saline to facilitate clearance of the drugs. Rats were allowed to recover for a minimum of 7 days before training.

2.3. Inhibitory avoidance

In order to habituate rats to the experimental procedures, they were handled for 2 min per day for five consecutive days before training. They were then trained on an inhibitory avoidance task. The inhibitory avoidance apparatus consisted of a trough-shaped alley (91 cm long, 15 cm deep, 20 cm wide at the top and 6.4 cm wide at the floor) that was divided into two compartments, separated by a manually controlled sliding door that opened by retracting into the floor. The starting compartment (31 cm long) was white and illuminated, whereas the shock compartment (60 cm long) was made of two dark electrifiable metal plates and was not illuminated. The rats were placed in the light "safe" compartment and allowed to cross to the dark "shock" compartment. After a rat stepped completely into the dark compartment, the sliding door was closed and a single inescapable footshock (0.32 mA, 1 s) was delivered. The rat was removed from the dark compartment 15 s later and, after drug treatment, returned to the home cage. Some animals received a retention test 48 h after training. During the retention test, rats were returned to the light compartment of the inhibitory avoidance apparatus and the latency to reenter the dark compartment with all four paws (maximum latency 600 s) was measured. Memory of the training experience was inferred from longer crossing latencies on the retention test. No shock or drug was delivered during retention testing. Other rats were sacrificed, either 15 min, 30 min, 45 min, or 1 h after training and drug treatment, and brains were used for analysis of Arc protein expression.

2.4. Microdialysis and HPLC

Norepinephrine levels in the amygdala were measured with *in vivo* microdialysis combined with high-performance liquid chromatography (HPLC) with coulometric detection (ESA Coulochem II,