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Corticosterone-induced enhancement of memory and synaptic Arc protein in the medial prefrontal cortex

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ABSTRACT

Acute administration of the stress hormone corticosterone enhances memory consolidation in a manner that is dependent upon the modulatory effects of the basolateral complex of the amygdala (BLA). Post-training administration of corticosterone increases expression of the activity-regulated cytoskeletal-associated protein (Arc) in hippocampal synaptic-enriched fractions. Interference with hippocampal Arc expression impairs memory, suggesting that the corticosterone-induced increase in hippocampal Arc plays a role in the memory enhancing effect of the hormone. Blockade of β-adrenoceptors in the BLA attenuates the corticosterone-induced increase in hippocampal Arc expression and blocks corticosterone-induced memory enhancement. To determine whether posttraining corticosterone treatment affects Arc protein expression in synapses of other areas of the brain that are involved in memory processing, a memory-enhancing dose of corticosterone was administered to rats immediately after inhibitory avoidance training. As seen in the hippocampus, Arc protein expression was increased in synaptic fractions taken from the prelimbic region of the medial prefrontal cortex (mPFC). Blockade of Arc protein expression significantly impaired memory, indicating that the protein is necessary in the mPFC for long-term memory formation. To test the hypothesis that blockade of β-adrenoceptors in the BLA would block the effect of systemic corticosterone on memory and attenuate mPFC Arc expression, as it does in the hippocampus, posttraining intra-BLA microinfusions of the β-adrenoceptor antagonist propranolol were given concurrently with the systemic corticosterone injection. Although this treatment blocked corticosterone-induced memory enhancement, it increased corticosterone-induced Arc protein expression in mPFC synaptic fractions. These findings suggest that the BLA mediates stress hormone effects on memory by participating in the negative or positive regulation of corticosterone-induced synaptic plasticity in efferent brain regions.

1. Introduction

Adrenal stress hormones modulate memory consolidation in human and non-human animals (Cahill, Prins, Weber, & McGaugh, 1994; de Quervain, Aerni, Schelling, & Roozendaal, 2009; Gold, van Buskirk, & McGaugh, 1975). Extensive evidence indicates that the basolateral complex of the amygdala (BLA) plays a critical role in this hormonal regulation of memory (McGaugh, 2004). For example, glucocorticoids interact with the noradrenergic system in the amygdala to enhance memories for emotionally arousing events in both humans and rats (Roozendaal, Barsegyan, & Lee, 2008; van Stegeren et al., 2007). In rodents, memory-enhancing corticosterone treatment increases norepinephrine levels in the amygdala (McReynolds et al., 2010). Studies performed in humans and rodents demonstrate that the amygdala interacts with multiple efferent brain regions, such as the hippocampus and medial prefrontal cortex (mPFC), during the memory consolidation period and this interaction is correlated with memory performance (Dolcos, LaBar, & Cabeza, 2004; Hayes et al., 2010; Murty, Ritchey, Adcock, & LaBar, 2010) for review see (McGaugh, 2004). A potential mechanism of BLA modulation of memory consolidation is through an influence on synaptic plasticity (Ikegaya, Saito, & Abe, 1994) and expression of plasticity-related proteins (Holloway-Erickson, McReynolds, & McIntyre, 2012; McIntyre et al., 2005) in efferent brain regions.

The protein product of the activity-regulated cytoskeletal-associated immediate early gene (*Arc*/*Arg* 3.1) is necessary for maintenance of hippocampal LTP and long-term memory of an aversive task (Guzowski et al., 2000; Holloway & McIntyre, 2011; McIntyre et al., 2005; Ploski et al., 2008). Our findings

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indicate that the BLA influences Arc protein expression in efferent brain regions. Posttraining intra-BLA infusions of the β-adrenergic agonist clenbuterol enhance memory and increase Arc protein expression in the hippocampus in a post-transcriptional manner (McIntyre et al., 2005). *Arc* mRNA is found in the stimulated regions of dendrites and can undergo local protein translation *in vitro*, suggesting regulation of Arc expression may occur at the synapse (Steward, Wallace, Lyford, & Worley, 1998; Yin, Edelman, & Vanderklish, 2002). Indeed, Arc protein expression is increased in dorsal hippocampal synapses when training on an aversive memory task is followed by memory-enhancing systemic injections of the stress hormone corticosterone. Antagonism of β-adrenoceptors in the BLA blocks corticosterone-induced enhancement of memory consolidation and attenuates the increase in hippocampal synaptic Arc protein expression (McReynolds et al., 2010). It is not yet determined that the role of the BLA as a mediator of stress hormone modulation of synaptic protein expression is conserved across brain regions.

The mPFC has a high density of glucocorticoid receptors (GRs) and has substantial anatomical connections with the BLA (McDonald, 1991; Meaney & Aitken, 1985; Reul & de Kloet, 1985). The prelimbic region of the mPFC is critically involved in the expression of conditioned fear and consolidation of aversive memories (Barsegyan, Mackenzie, Kurose, McGaugh, & Roozendaal, 2010; Corcoran & Quirk, 2007). Infusions of a GR agonist into either the BLA or the mPFC enhance the consolidation of long-term memory whereas inhibiting the MAPK cascade in either region prevents the memory-enhancing effect of a GR agonist infused into the other. This suggests that the mPFC and BLA must function as a circuit to modulate memory consolidation (Roozendaal et al., 2009).

If the role of the BLA as a mediator of stress hormone modulation of synaptic protein expression is conserved across brain regions, memory-enhancing glucocorticoids should exert their effects through noradrenergic actions in the BLA which, in turn, increase synaptic plasticity-associated proteins such as Arc in regions of the brain that support long-term memory. According to evidence that the prelimbic (PL) region of the mPFC is critically involved in the consolidation of conditioned fear and aversive memory, memory-enhancing corticosteroid administration should increase expression of Arc protein in synapses of the PL and inactivation of the noradrenergic system within the BLA should attenuate that Arc effect. The present study examined the effect of posttraining administration of corticosterone on memory and synaptic Arc protein expression in synaptoneurosomes taken from the rat mPFC. In order to determine whether Arc protein expression in the mPFC was a critical component of memory consolidation, *Arc* translation to protein was blocked with intra-mPFC microinfusions of Arc antisense oligodeoxynucleotides. Finally, to test the hypothesis that BLA norepinephrine interacts with corticosterone-induced Arc protein expression in the mPFC, intra-BLA infusions of the β-adrenoceptor antagonist propranolol were administered immediately following training and administration of corticosterone. In order to target the consolidation phase of memory processing while avoiding performance effects, all interventions were given immediately after training.

2. Materials and methods

2.1. Subjects

Two hundred and two male Sprague–Dawley rats (250–275 g upon arrival), purchased from Charles River Breeding Laboratories, 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 one 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 Texas at Dallas).

2.2. Surgery

For implantation of infusion guide cannulae, animals were anesthetized with isoflurane (1% in O_2) (Western Medical Supply) and the skull was positioned in a stereotaxic frame (Stoelting Inc). For animals used in the intra-BLA infusion experiment, two 15-mm-long guide cannulae (23 gauge; Small Parts) were implanted bilaterally with the tips 2 mm above the BLA [coordinates: anteroposterior (AP), −2.7 mm from Bregma; mediolateral (ML), ±5.2 mm from midline; dorsoventral (DV), −6.4 mm below skull surface; incisor bar, −3.3 mm from interaural line (Paxinos & Watson, 2005)]. 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. For animals used in the intra-mPFC infusion experiment, two 12-mm-long guide cannulae (23 gauge; Small Parts) were implanted bilaterally with the tips 1.5 mm above the prelimbic region of the mPFC (AP, +3.6 mm; ML, ±0.5 mm; DV, −2.5 mm). The guide cannulae were fixed in place with acrylic dental cement and two small anchoring screws. Stylets (12-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, 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. This footshock value was chosen because it does not increase norepinephrine levels in the BLA. However, a significant elevation of norepinephrine and memory enhancement were observed when IA training with a 0.32 mA footshock was paired with an immediate posttraining systemic injection of corticosterone (3 mg/kg, i.p.; McReynolds et al., 2010). For rats that were used in the intra-BLA infusion experiment, the footshock value was 0.38 mA for 1 s. For rats that were used in the intra-mPFC infusion experiment, the footshock value was 0.45 mA for 1 s to ensure that the control animals showed sufficient memory. Rats were removed from the dark compartment 15 s after footshock administration and, after drug treatment, returned to the home cage. Some rats were given 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 animals were sacrificed 15 min,

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