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Research Report

Interplay between serotonin and cannabinoid function in the amygdala in fear conditioning

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ABSTRACT

The possible interactions between the cannabinoid and serotonin systems in the regions of the brain involved in emotional learning and memory formation have been studied by some researchers. In view of the key role of the amygdala in the acquisition and expression of fear memory, we investigated the involvement of basolateral amygdala (BLA) serotonin 5-HT4 receptors in arachidonylcyclopropylamide (ACPA; selective CB1 cannabinoid receptor agonist)-induced fear memory consolidation impairment. In our study, a context and tone fear conditioning apparatus was used for testing fear conditioning in adult male NMRI mice. The results showed that intraperitoneal administration of ACPA 0.5 or 0.05, 0.1 and 0.5 mg/kg immediately after training decreased the percentage of freezing time in context or tone fear conditioning respectively, suggesting a context- or tone-dependent fear memory consolidation impairment. Post-training intra-BLA microinjections of RS67333, as 5-HT4 serotonin receptor agonist, at doses of 0.025 and 0.05 μg/mouse also impaired context or tone memory consolidation, while RS23597, as 5-HT4 serotonin receptor antagonist, did not produce a marked difference in both fear memories as compared with the control group. Moreover, a subthreshold dose of RS67333 did not alter ACPA response in both fear conditionings. Interestingly, a subthreshold dose of RS23597 potentiated or reversed ACPA response at the dose of 0.01 or 0.05 respectively. It is concluded that BLA serotonin 5-HT4 receptors are involved in tone-dependent fear memory consolidation impairment induced by CB1 activation using ACPA, suggesting a modulatory role for serotonin 5-HT4 receptor.

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1. Introduction

The amygdala has long been known to play a key role in supporting memory for emotionally arousing experiences (Hermans et al., 2014). Neuroanatomical and the molecular basis of emotional learning and memory have been widely studied by Pavlovian fear conditioning. Sound, light or a specific context can be paired with foot shock as a neutral conditioned stimulus which will elicit a conditioned fear response in the absence of unconditioned stimulus. Conditioned context- and tone-dependent fear can be acquired in a single trial and can be studied independent of one another (Misane et al., 2013).

Although different areas of the brain have been suggested to be involved in fear conditioning, the amygdala may be the main control center for the acquisition and expression of auditory fear conditioning (Gale et al., 2004; Phelps and LeDoux, 2005; Rodrigues et al., 2004). It has been shown that fear learning depends on the induction of neural plasticity within the basolateral complex of the amygdala (BLA) (Miserendino et al., 1990; Rogan et al., 1997). The BLA receives sensory projections (Luskin and Price, 1983; Miki et al., 1998) from other brain regions that encode information about conditioned stimuli (CSs) and unconditioned stimuli (USs). The information, after entering the BLA, is transferred to the central amygdala (CeA) for output (Sah et al., 2003). The BLA plays a selective role in memory consolidation in a variety of training tasks, such as contextual fear conditioning (LaLumiere et al., 2003) and cued fear conditioning (Schafe and LeDoux, 2000).

Ample evidence indicates that the BLA expresses high densities of cannabinoid CB1 receptors (Campolongo et al., 2009). The endocannabinoid system has an essential role for the modulation of emotional conditions and also reducing the fear-conditioning phenomenon (Kamprath and Wotjak, 2004; Marsicano et al., 2002). Moreover, increased endocannabinoid signaling decreases fear-conditioned response in different behavioral tasks (Bitencourt et al., 2008; Chhatwal et al., 2005; Lin et al., 2009; Pamplona et al., 2008). In a fear conditioning study, it has been shown that cannabinoidergic agents in the BLA can influence the various phases of learning and memory; For example, WIN 55, 212-2, as a CB1R agonist, reduces fear consolidation in the BLA (Kuhnert et al., 2013).

There are many studies supporting the idea that the endocannabinoid signaling modulates the behavioral responses, at least in part, via the regulation of the serotonin (5-HT) system (Haj-Dahmane and Shen, 2011). It has been suggested that endocannabinoids reduce 5-HT release in the amygdala- known to be specifically involved in mediating fear- via the activation of CB1 receptors (Ashton et al., 2006). The expression of 5-HT4 receptors in limbic areas, including amygdala, is highly suggestive of a role for these receptors in emotional processes (Waeber et al., 1994). In addition, 5-HT4 receptor expression changes in diverse brain areas during learning and memory tasks (Manuel-Apolinar et al., 2005). Cumulative evidence indicates that 5-HT4 receptor agonists modulate synaptic plasticity within the hippocampus and amygdala by augmenting long-term potentiation, attenuating depotentiation and altering patterns of long-term depression (Huang and Kandel, 2007; Kemp and Manahan-Vaughan, 2005). Our previous investigations showed that serotoninergic 5-HT4 receptors are involved in the responses induced by the activation of CB1 cannabinoid receptors (e.g. anxiety-related behaviors and emotional learning and memory formation) in the different brain regions such as ventral hippocampus (Nasehi et al., 2015b), nucleus accumbens (Khodayar et al., 2015) and BLA (Chegini et al., 2014). Considering the critical role of the BLA in emotional memory formation and in view of the fact that there is an interaction between functions of cannabinoid and 5-HT receptors and availability of 5-HT4 agents, the present study was designed to evaluate the role of serotonin 5-HT4 receptors of the BLA in mediating ACPA fear memory consolidation impairment. Thus, we studied whether ACPA-induced memory consolidation impairment could be affected by intra-BLA microinjections of serotonin 5-HT4 receptor agonist and/or antagonist.

2. Results

2.1. *Effect of RS67333 or RS23597 microinjection into the BLA on context- and tone-dependent memory consolidation*

Fig. 1A–D shows the effect of post-training intra-BLA microinjection of RS67333 or RS23597 on context-dependent memory consolidation. One-way ANOVA showed that RS67333 [$F(3, 28)=21.75$, $P<0.001$] but not RS23597 [$F(3, 28)=2.99$, $P>0.05$] impaired context-dependent fear memory consolidation. In addition, one-way ANOVA illustrated that RS67333 produced a significant difference in latency [$F(3, 28)=35.01$, $P<0.001$ and % rearing [$F(3, 28)=3.71$, $P<0.05$] but not % grooming [$F(3, 28)=1.92$, $P>0.05$]. Similar analysis showed that RS23597 induced a significant difference in latency [$F(3, 28)=8.69$, $P<0.001$ and % grooming [$F(3, 28)=16.69$, $P<0.001$] but not % rearing [$F(3, 28)=0.49$, $P>0.05$]. As determined by Tukey's post-hoc comparisons, RS67333 (0.025 and 0.05 μg/mouse) impaired context-dependent memory consolidation and decreased latency to the freezing, while RS23597 (0.025 μg/mouse) or RS23597 (0.025 and 0.05 μg/mouse) increased % grooming or latency to the freezing respectively.

Fig. 1E–H shows the effect of post-training intra-BLA microinjection of RS67333 or RS23597 on tone-dependent memory consolidation. One-way ANOVA indicated that RS67333 [$F(3, 28)=34.05$, $P<0.001$] but not RS23597 [$F(3, 28)=1.57$, $P>0.05$] impaired tone-dependent fear memory consolidation. In addition, one-way ANOVA revealed that RS67333 produced a significant difference in latency [$F(3, 28)=38.64$, $P<0.001$] and % rearing [$F(3, 28)=5.12$, $P<0.01$] but not % grooming [$F(3, 28)=1.78$, $P>0.05$]. Similar analysis showed that RS23597 induced a significant difference in latency [$F(3, 28)=6.45$, $P<0.01$] and % rearing [$F(3, 28)=5.39$, $P<0.01$] but not % grooming [$F(3, 28)=0.45$, $P>0.05$]. As determined by Tukey's post-hoc comparisons, RS67333 (0.025 and 0.05 μg/mouse) impaired tone-dependent memory