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The effects of enhancing endocannabinoid signaling and blocking corticotrophin releasing factor receptor in the amygdala and hippocampus on the consolidation of a stressful event

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Abstract
Current clinical and pre-clinical data suggest that both cannabinoid agents and blockage of CRF through corticotrophin releasing factor receptor type 1 (CRFr1) may offer therapeutic benefits for post-traumatic stress disorder (PTSD). Here we aim to determine whether they are more effective when combined when microinjected into the basolateral amygdala (BLA) or CA1 area of the hippocampus after exposure to a stressful event in the shock/reminders rat model for PTSD. Injection of the fatty acid amide hydrolase (FAAH) inhibitor URB597 after the shock into either the BLA or CA1 facilitated extinction, and attenuated startle response and anxiety-like behavior. These preventive effects of URB597 were found to be mediated by the CB1 receptor.
Intra-BLA and intra-CA1 microinjection of the CRFr1 antagonist, CP-154,526 attenuated startle response. When microinjected into the BLA, CP-154,526 also attenuated freezing behavior during exposure to the first reminder and decreased anxiety-like behavior. The combined treatment of URB597 and CP-154,526 was not more effective than the separate treatments.
Finally, mRNA levels of CRF, CRFr1 and CB1r were significantly higher in the BLA of rats exposed to shock and reminders compared to non-shocked rats almost one month after the shock.
Taken together, the results show that enhancing endocannabinoid signaling in the amygdala and hippocampus produced a more favorable spectrum of effects than those caused by the CRFr1 antagonist. The findings suggest that FAAH inhibitors may be used as a novel treatment for stress-related anxiety disorders.

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1. Introduction

Exposure to an excessive or an uncontrolled stress is a major factor associated with various diseases including post-traumatic stress disorder (PTSD). The consequences of exposure to trauma are affected not only by aspects of the event itself, but also by the frequency and severity of trauma reminders.

Several lines of evidence support the role of the endocannabinoid (eCB) system as a modulator of the hypothalamic pituitary adrenal (HPA) axis (Akirav, 2013; Haring et al., 2012; Patel et al., 2004) and of the behavioral responses to stress, including anxiety-related behaviors, and extinction of fear memories (Ganon-Elazar and Akirav, 2012; Steiner et al., 2008). Recent clinical (Fraser, 2009; Hauer et al., 2013; Roitman et al., 2014) and preclinical studies pointed the eCB system as a possible therapeutic target to treat both the emotional and cognitive dysfunctions characterizing PTSD (Ganon-Elazar and Akirav, 2012; Trezza and Campolongo, 2013). The eCB system includes the cannabinoid receptors (CB1r and CB2r), eCBs (N-arachidonylethanolamine [AEA/anandamide] and 2-arachidonoyl-glycerol [2-AG]), enzymes involved in their synthesis and metabolism (fatty acid amide hydrolase (FAAH) for AEA and the monoacylglycerol lipase (MAGL) for 2-AG), and an eCB transporter.

The FAAH inhibitor URB597 magnifies AEA-mediated CB1r signaling and produce a more circumscribed and beneficial spectrum of biological effects than those caused by direct CB1r activation (Piomelli, 2005). FAAH inhibitors were found to disrupt contextual fear conditioning (Burman et al., 2016) and to facilitate extinction of contextual fear and a spatial task (Laricchiuta et al., 2013; Varvel et al., 2007). Furthermore, the FAAH inhibitor AM3506 microinjected into the BLA before extinction training was shown to facilitate extinction and this effect was CB1r-dependent (Gunduz-Cinar et al., 2013). We found that systemic administration of URB597 after exposure to SPS prevented the SPS-induced impairment in extinction and hippocampal plasticity (Zer-Aviv and Akirav, 2016).

The CRF1 receptor (CRFr1) has been demonstrated as a potential drug target for antidepressants and anxiolytics in animals (Adamec et al., 2010; Overstreet and Griebel, 2004; Philbert et al., 2012). The CRFr1 is a key player in the response to stress and it was notably observed that PTSD patients exhibit increased CSF levels of the endogenous peptide, CRF (Baker et al., 1999; Bremner et al., 1997), suggesting dysregulation of CRF may contribute to PTSD (Gafford and Ressler, 2015).

CB1r and CRFr1 are widely expressed in the hippocampus and BLA (Herkenham et al., 1991; Chen et al., 2000), two brain regions that are involved in fear and memory and are highly implicated in PTSD. Two recent studies pointed on a possible eCB–CRF interaction in the amygdala (Gray et al., 2015; Natividada et al., 2017) and suggested that CRF signaling coordinates a disruption of tonic AEA activity to promote a state of anxiety. Gray et al. (2015) showed that CRF, through activation of CRFr1, evoked a rapid induction of FAAH, which caused a reduction in AEA, within the amygdala. Also, CRF signaling in the amygdala promoted an anxious phenotype that was prevented by FAAH inhibition. Natividada et al. (2017) showed that genetically-selected Marchigian Sardinian P rats carrying an innate overexpression of CRFr1 exhibited lower concentrations of AEA in the amygdala in association with increases in amygdalar FAAH activity and anxious phenotype.

Here we aimed to compare the effects of the FAAH inhibitor URB597 and the CRFr1 antagonist CP-154,526 in the BLA and CA1 on the consolidation of a traumatic event in rats exposed to the shock and reminders model of PTSD. To that end, rats were microinjected with the FAAH inhibitor or the CRFr1 antagonist in the BLA and CA1 immediately after the shock and tested for fear and anxiety-related symptoms at least a month later. In this PTSD model, rats are exposed to a single footshock in an inhibitory avoidance apparatus followed by contextual situational reminders (SRs) of the shock. Previous studies with this model demonstrated that mice showed increased anxiety-like behaviors, heightened startle reflexes, learned helplessness, and impaired social behavior (Louvart et al., 2005; Pynoos et al., 1996; Siegmund and Wotjak, 2007). We found that rats exposed to shock and reminders showed impaired fear extinction, impaired plasticity in the hippocampal-accumbens pathway, and enhanced latency to startle (Korem and Akirav, 2014).

Hence, we examined the long-lasting effects of exposure to shock and reminders on extinction, startle response, anxiety-like behavior and gene expression of CRFr1 and CB1r in the amygdala and hippocampus. Importantly, we aimed to compare the separated and combined effects of blocking CRF signaling and enhancing eCBs on the consolidation of an emotional traumatic memory.

2. Experimental procedures

2.1. Subjects

Males Sprague-Dawley rats (220–250 g; Harlan, Jerusalem, Israel) were caged together (2–5 per cage) at 22 $\pm$ 2 °C under 12-h light/dark cycles (lights turned on at 7am). Rats were allowed water and laboratory rodent chow *ad libitum*. The experiments were approved by the University of Haifa Ethics and Animal Care Committee, and adequate measures were taken to minimize pain or discomfort.

2.2. Acoustic startle response (ASR)

The rats were placed in 8 $\times$ 8 $\times$ 16 cm open animal holder that restricted locomotion but did not immobilize the animal (Coulbourn Instruments). Chambers were calibrated for both sensitivity to movement and sound level to ensure consistency between chambers and experiments. The animals were placed in the holder and allowed a 5-min acclimatization period, 30 acoustic startle trials (98 or 120 dB; 50 ms duration, 20–40 s intertrial interval) were presented over the 68 dB white noise background. The mean startle amplitude was assessed. Mean startle amplitude indicates the average of the response to the 98 and 120 dB. The startle response to both stimulus intensities was averaged as there were no selective effects on one or the other. Acoustic startle response (ASR) was tested before the shock (ASR1) and after the last extinction trial (ASR2).

2.3. Inhibitory avoidance stress

Rats were placed in an inhibitory avoidance apparatus that consists of a box (50 cm $\times$ 25 cm $\times$ 30 cm), divided into two equal size