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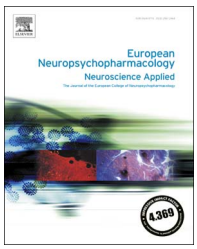

Chronic treatment with URB597 ameliorates post-stress symptoms in a rat model of PTSD

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Abstract
Activating the endocannabinoid system has become a major focus in the search for novel therapeutics for anxiety and deficits in fear extinction, two defining features of PTSD. We examined whether chronic treatment with the fatty acid amide hydrolase (FAAH) inhibitor URB597 (0.2, 0.3, 0.4 mg/kg, i.p.) or the CB1/2 receptor agonist WIN55,212-2 (0.25, 0.5 mg/kg, i.p.) injected for 3 weeks to rats exposed to the shock and reminders model of PTSD would attenuate post-stress symptoms and affect basolateral amygdala (BLA) and CA1 CB1 receptors. Exposure to shock and reminders enhanced acoustic startle response and impaired extinction. Rats exposed to shock and reminders and chronically treated with URB597 demonstrated normalized startle response and intact extinction kinetics. WIN55,212-2 only affected the startle response. The therapeutic effects of URB597 and WIN55,212-2 were found to be CB1 receptor dependent, as these effects were blocked when a low dose of the CB1 receptor antagonist AM251 (0.3 mg/kg, i.p. for 3 weeks) was co-administered. Moreover, URB597, but not WIN55,212-2, normalized the shock/reminders-induced upregulation in CB1 receptor levels in the BLA and CA1. One hour after the shock, *N*-arachidonoylethanolamine (AEA) was increased in the BLA and decreased in the CA1. Circulating 2-arachidonoylglycerol (2-AG) concentrations were decreased in shocked rats, with no significant effect in the BLA or CA1. FAAH activity was increased in the CA1 of shocked rats. Chronic cannabinoid treatment with URB597 can ameliorate PTSD-like symptoms suggesting FAAH inhibitors as a potentially effective therapeutic strategy for the treatment of disorders associated with inefficient fear coping.

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1. Introduction

The formation of a fear memory following a traumatic event is an important mechanism for the subsequent development of posttraumatic stress disorder (PTSD). Impaired extinction of fear memories is thought to contribute to the development and persistence of anxiety disorders including PTSD (Charney et al., 1993; Lissek et al., 2005; Milad et al., 2007). Indeed, central clinical features of PTSD are the persistence of a heightened salience of traumatic memories (i.e. experience of alarm and distress) and a failure of the extinction process to diminish the impact of traumatic memories (Rothbaum and Davis, 2003). We have recently shown that exposure to shock and reminders model of PTSD, in which rats are exposed to a severe footshock in an inhibitory avoidance apparatus followed by exposure to situational reminders (SRs), results in long-term impairment in extinction and enhancement of the startle response (Aisenberg et al., 2017; Shoshan and Akirav, 2017; Shoshan et al., 2017).

Two brain regions that are involved in fear and memory and are highly implicated in PTSD are the hippocampus and amygdala; both regions participate simultaneously in the early stages of memory formation and in the retrieval of inhibitory avoidance (Bianchin et al., 1993; Izquierdo et al., 1992, 1993). However, the basolateral amygdala (BLA) is involved in the acquisition of extinction (Berlau and McGaugh, 2006) and the hippocampus is involved in contextual modulation of extinction (Ji and Maren, 2007).

Several lines of evidence support the role of the endocannabinoid (eCB) system in the modulation of cognitive functions and the stress response and it has been suggested as a therapeutic target for the treatment of severe stress associated with PTSD (Ganon-Elazar and Akirav, 2012; Hillard, 2014; Lutz et al., 2015; Marsicano et al., 2002; Moreira and Wotjak, 2010). Recent clinical (Fraser, 2009; Hauer et al., 2013; Roitman et al., 2014) and preclinical studies (Ganon-Elazar and Akirav, 2012; Hill et al., 2018; Trezza and Campolongo, 2013) implicate the eCB system as a possible therapeutic target to treat both the emotional and cognitive dysfunctions characterizing PTSD. In support of this notion, individuals with PTSD are more likely to use cannabis to help with symptoms coping (Betthauser et al., 2015; Bonn-Miller et al., 2007).

The eCB system consists of cannabinoid receptors (CB1r and CB2r), their endogenous lipid ligands eCBs (*N*-arachidonoylethanolamine/anandamide [AEA] and 2-arachidonoylglycerol [2-AG]), and the enzymatic machinery for eCB synthesis and degradation, including the AEA and 2-AG hydrolytic enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. Genetic and pharmacological blockade of CB1r signaling were found to impair fear extinction (Marsicano et al., 2002; Chhatwal et al., 2005; Abush and Akirav, 2010; Ganon-Elazar and Akirav, 2009) whereas enhancing eCB signaling has been shown to facilitate extinction in various studies (Abush and Akirav, 2010; De Oliveira Alvares et al., 2008; Do Monte et al., 2013; Lin et al., 2009). PTSD patients demonstrate elevated CB1r binding sites in the amygdala-hippocampal-cortico-striatal neural circuit (Neumeister et al., 2013). In animal models, we have recently found upregulation of CB1r in the BLA and CA1 after exposure to shock and reminders model of PTSD (Aisenberg et al., 2017; Shoshan et al., 2017; Shoshan and Akirav, 2017).

Studies from our lab have demonstrated that acutely enhancing eCB signaling using the fatty acid amide hydrolase (FAAH) inhibitor URB597 (URB) or activating CB1 receptors (CB1r) using the CB1/2 receptor agonist WIN55,212-2 (WIN), after exposure to severe stress, prevented the development of stress-related symptoms such as impaired extinction, enhanced acoustic startle response, and altered plasticity in the hippocampus, BLA and nucleus accumbens (NAc) (Aisenberg et al., 2017; Korem and Akirav, 2014; Shoshan and Akirav, 2017; Shoshan et al., 2017; Zer-Aviv and Akirav, 2016). Moreover, we have recently found that WIN and URB not only impaired the formation of inhibitory avoidance memory (i.e., associative memory), but also of hyperarousal (i.e., non-associative memory) at the level of the CA1 region and the BLA in rats exposed to the shock and reminders model (Aisenberg et al., 2017; Shoshan and Akirav, 2017; Shoshan et al., 2017). However, the effects of chronically administered cannabinoid agonists have not yet been demonstrated in this model.

There are reports that the onset of PTSD symptoms following exposure to a traumatic event triggers cannabis use as a means of self-medication (Betthauser et al., 2015; Bonn-Miller et al., 2007). Hence, in the current study, cannabinoid agents were administered chronically after symptoms develop (Korem and Akirav, 2014). The aim was to examine whether chronic treatment with URB or WIN would prevent the long-term effects of exposure to severe stress on fear retrieval, extinction and startle response, and the involvement of CB1r in the BLA and CA1. We chose relatively low doses of cannabinoids as there are numerous of studies suggesting that cannabinoids could produce anxiety that likely depend on the doses used, with higher doses potentiating stress- and anxiety-like responses (Lutz, 2009; Rodriguez de Fonseca et al., 1996; Scherma et al., 2008; Viveros et al., 2005). We also aimed at examining the effects of shock exposure on the endogenous cannabinoid system due to the centrality of the trauma (the shock in our model) as a trigger to post-trauma symptoms.

2. Experimental procedures

2.1. Animals

Male Sprague-Dawley rats (60 days old, ~250 g; Harlan, Jerusalem, Israel) were grouped housed at 22±2 °C under 12-h light/dark cycles (lights turned on at 07:00). The experiments started after one week of habituation to the animal room, hence rats were exposed to shock on postnatal day (P)67 and the first injection was on P73. Rats were allowed water and laboratory rodent chow ad libitum. All experiments were approved by the University of Haifa Ethics and Animal Care Committee, and adequate measures were taken to minimize pain or discomfort (307/14).

2.2. Drug treatment

URB597 (0.2, 0.3, or 0.4 mg/kg, i.p.), WIN55,212-2 (0.25 or 0.5 mg/kg, i.p.) and AM251 (0.3 mg/kg, i.p.) (Cayman Chemicals) were dissolved in 10% dimethylsulfoxide (DMSO), 5% Tween-80, 1%

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