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Endocannabinoid concentrations in hair are associated with PTSD symptom severity

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ABSTRACT

The endocannabinoid system has been implicated in the regulation of the stress response, fear memory formation, and inflammatory processes. Posttraumatic stress disorder (PTSD) can result from exposure to extreme stress and is characterized by strong, associative memories for the traumatic events experienced. Furthermore, an elevated physical disease risk has been observed in PTSD, likely to be mediated by inflammatory processes. Therefore, altered endocannabinoid regulation can be expected in individuals with PTSD. However, attempts to assess PTSD-associated differences in the endocannabinoid system from human blood samples have provided inconsistent results, possibly due to fluctuating levels of endocannabinoids. In hair, these neuromodulators are accumulated over time and thus give access to a more stable and reliable assessment.

We therefore investigated PTSD-associated differences in hair concentrations of endocannabinoids (*N*-acyl-ethanolamides palmitoylethanolamide [PEA], oleoylethanolamide [OEA] and stearoylethanolamide [SEA]) in 38 rebel war survivors from Northern Uganda suffering from PTSD and $N = 38$ healthy rebel war survivors without current and lifetime PTSD. PTSD diagnosis and symptom severity were assessed in structured clinical interviews employing the Posttraumatic Diagnostic Scale (PDS). A significant group difference was observed for OEA, with PTSD patients showing reduced hair concentrations. Regression analyses further revealed strong negative relationships between all investigated *N*-acyl-ethanolamides and symptom severity of PTSD. The observed reductions in endocannabinoids might account for the increased inflammatory state as well as for the failure to extinguish fear memories observed in PTSD. Our findings add to the accumulating evidence suggesting the endocannabinoid system as a target for pharmacological enhancement of exposure-based psychotherapy for PTSD.

1. Introduction

Posttraumatic stress disorder (PTSD) is a severe mental health disorder that can occur after extreme stress, such as war, rape or torture. Memory alterations represent the most characteristic feature of PTSD: While the traumatic events are re-experienced with high emotional and sensory intensity and vividness, the corresponding context information and chronological order is often difficult to recall (Brewin, 2015). In addition, PTSD is associated with an elevated risk for cardiovascular, autoimmune, metabolic and inflammatory diseases (Boscarino, 2004; Glaesmer et al., 2011; Roberts et al., 2015; Rosenbaum et al. 2015a; Seng et al., 2006).

The endocannabinoid system (ECS) comprises endogenous cannabinoid receptors and endocannabinoid neurotransmitters. The most extensive investigated endocannabinoids are anandamide (AEA) and 2-arachidonoylglycerol (2-AG) which both bind to CB1 and CB2 receptors. Other biologically highly active molecules linked to the ECS, which include *N*-acyl-ethanolamides such as palmitoylethanolamide (PEA), oleoylethanolamide (OEA) and stearoylethanolamide (SEA), are structurally highly similar to AEA and can potentiate its effects (Ho et al., 2008; Jonsson et al., 2001). Recently, PTSD research has focused on the ECS due to its role in stress regulation, memory processes, and inflammation (Neumeister et al., 2015).

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Animal and human research indicates a central role of endocannabinoids and related *N*-acyl-ethanolamides in regulating the HPA axis and limiting stress reactions, as well as in the adaption to chronic stress. In humans, peripheral serum concentrations of the endogenous cannabinoid AEA and the related *N*-acyl-ethanolamides PEA and OEA were found to be elevated in the immediate aftermath of a social stressor (Dlugos et al., 2012), but declined in the subsequent recovery phase (Hill et al., 2009). Animal research has repeatedly shown a decrease in AEA in almost all brain regions investigated in response to both acute and chronic stress, which seems to contribute to HPA axis activation, as well as an anxiety phenotype (Morena et al., 2015). Furthermore, a peripheral decline of the *N*-acylethanolamides PEA and OEA in the aftermath of predator stress was observed in animals, too (Holman et al., 2014). Correspondingly, knockout of endocannabinoid receptors was associated with impaired stress coping and enhanced anxiety and depressive-like symptoms, while stimulation of the ECS reduced anxiety and stress-related behavioral impairments in stress-exposed rats (reviewed in Hill and Patel (2013)).

Since PTSD is characterized by strong associative fear memories and a failure to extinguish fear responses to trauma reminders, fear conditioning research represents an important translational model for the disorder (Amstadter et al., 2009). Disruption of endocannabinoid signaling by peripheral administration of cannabinoid receptor antagonists or genetic knockout of cannabinoid receptors leads to impairment in extinction learning in animals (see Papini et al. (2015) for a systematic review). Furthermore, there is compelling evidence indicating that peripheral stimulation of the ECS e.g., by systemically administrating an inhibitor of fatty acid amide hydrolase, the most important enzyme for the degradation of AEA, PEA, OEA, and SEA, facilitates extinction learning and retention of extinction memory in both animals and humans (de Bitencourt et al., 2013; Papini et al., 2015).

Finally, endocannabinoids and related *N*-acyl-ethanolamides exert anti-inflammatory effects (Sayd et al., 2015). Taken together, the summarized literature strongly suggests that reduced endocannabinoid signaling in PTSD, a condition of chronic stress, could contribute to the explanation of the strong difficulties to extinguish fear reactions, the anxiety phenotype, as well as the state of low-grade inflammation observed in PTSD. Accordingly, the ECS has been proposed to play a significant role in PTSD etiology, and could represent a pharmacological treatment option for PTSD (Neumeister et al., 2015).

Indeed, reduced relative PEA concentrations were the strongest hallmark of PTSD in a metabolite profiling study (Karabatsiakis et al., 2015). Further studies also point towards reduced concentrations of some endocannabinoids and related *N*-acyl-ethanolamides in PTSD (Hill et al., 2013; Neumeister et al., 2013). However, opposite or null effects have also been reported (Hauer et al., 2013; Schaefer et al., 2014). These inconsistencies might be due to strong variations of plasma endocannabinoid concentrations during the day (Vaughn et al., 2010). In particular in PTSD, recent intrusive symptoms might trigger the stress response and lead to daily variations in endocannabinoid concentrations.

The analyses of hair as opposed to blood, saliva or urine has long been used in toxicology, doping and forensic sciences. Substances (e.g., drugs of abuse or endogenous substances) can be incorporated into hair by different ways. Mainly discussed is the diffusion from the blood stream during formation of the hair shaft and the absorption after formation by sweat and sebum (Henderson, 1993). The advantages of hair analyses include high reliability, reduced bias by daily variations and the possibility of retrospective assessments over several months. The work group of Kirschbaum et al. (2009) introduced the idea that hair analyses might be useful to analyze the effects of psychological stress over longer time periods on stress hormones like glucocorticoids in human noninvasive studies. They were able to show that hair cortisol levels in pregnant women reflected the alterations of cortisol levels observed in peripheral blood during pregnancy (Kirschbaum et al., 2009). In a previous validation study, we observed that endocannabinoids tend to accumulate in hair during pregnancy (Krumbholz et al., 2013) and that this accumulation process can probably be compared to that seen with cortisol (Kirschbaum et al., 2009). The assessment of endocannabinoid concentrations in hair might hence represent a more reliable and stable assessment of the endocannabinoid system (Krumbholz et al., 2013) which could be especially useful for PTSD research.

Here, we present the results from the first study employing this novel method to investigate PTSD-associated endocannabinoid levels in hair samples of severely traumatized rebel war survivors from Northern Uganda. In line with the literature reviewed above, we hypothesized that hair concentrations of endocannabinoids/*N*-acyl-ethanolamides would be reduced in individuals with PTSD and correlate negatively with PTSD symptom severity.

2. Material and methods

2.1. Study participants

Analyses were based on a sample of $N = 76$ survivors of the war between the rebel group Lord's Resistance Army (LRA) and the Ugandan Governmental forces from Northern Uganda. The recruitment took place in the former internal displaced people (IDP) camps Pabbo, Koch Goma and Pakiri in Northern Uganda. Initially, the study procedures were explained to the local leaders and community members in community meetings. During the data collection phase, trained local interviewers approached the clients in their homes, and invited one adult family member per household who was affected by the LRA conflict to participate. If more than one family member volunteered to participate, the most affected person was chosen, in order to recruit a sufficient number of current PTSD cases into the study.

The PTSD group consisted of $N = 38$ LRA war survivors with a diagnosis of current PTSD according to DSM-IV, and a current symptom score ≥ 10 who had never received any psychotherapeutic or medical treatment for their PTSD symptoms at the time of investigation. The control group comprised $N = 38$ war survivors with the absence of a current, as well as a lifetime PTSD diagnosis. The groups were matched with respect to age and gender. Since both groups were recruited in the same former IDP camps, they faced similar living conditions. At the time of the assessment, the former camps were safe as rebel attacks within Northern Uganda had stopped since the ceasefire agreement in 2005. However, both groups faced daily stressors, which included poverty, and insufficient access to education or medical care.

2.2. Clinical assessment

Trained local interviewers performed the diagnostic interviews under the supervision of clinical psychologists specialized in traumatic stress studies from the universities of Ulm and Konstanz, Germany. The interviewers attended a six-week in-depth training on the concepts of quantitative data collection and mental health disorders focusing on the clinical assessment of traumatic event exposure and PTSD. All study instruments were translated into the local language, Luo. Translations were followed by blind back-translations, and group discussions with independent interpreters, to ensure a valid translation of the instruments.

A 62-item event-list was employed to assess the number of traumatic event types experienced. This event-list was based on previous investigations of our work group in Sub-Saharan Africa