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Anandamide reverses depressive-like behavior, neurochemical abnormalities and oxidative-stress parameters in streptozotocin-diabetic rats: Role of CB1 receptors

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Abstract
The pathophysiology associated with increased prevalence of depression in diabetics is not completely understood, although studies have pointed the endocannabinoid system as a possible target. Then, we aimed to investigate the role of this system in the pathophysiology of depression associated with diabetes. For this, diabetic (DBT) male Wistar rats were intraperitoneally treated with cannabinoid CB1 (AM251, 1 mg/kg) or CB2 (AM630, 1 mg/kg) receptor antagonists followed by anandamide (AEA, 0.005 mg/kg) and then submitted to the forced swimming test (FST). Oxidative stress parameters, CB1 receptor expression and serotonin (5-HT) and noradrenaline levels in the hippocampus (HIP) and prefrontal cortex (PFC) were also performed. It was observed that DBT animals presented a more pronounced depressive-like behavior and increase of CB1 receptor expression in the HIP. AEA treatment induced a significant improvement in the depressive-like behavior, which was reversed by the

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CB1 antagonist AM251, without affecting the hyperglycemia or weight gain. AEA was also able to restore the elevated CB1 expression and also to elevate the reduced level of 5-HT in the HIP from DBT animals. In addition, AEA restored the elevated noradrenaline levels in the PFC and induced a neuroprotective effect by restoring the decreased reduced glutathione and increased lipid hydroperoxides levels along with the decreased superoxide dismutase activity observed in HIP or PFC. Together, our data suggest that in depression associated with diabetes, the endocannabinoid anandamide has a potential to induce neuroadaptative changes able to improve the depressive-like response by its action as a CB1 receptor agonist.

1. Introduction

Diabetes *mellitus* (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from failure in insulin secretion and/or in its action (Brownlee, 2005; Wayhs et al., 2010; Zanoveli et al., 2015). It is known that the damage caused by hyperglycemia includes dysfunction and failure of various organs being the brain one of the most affected organs (Sevick et al., 2007; Moulton et al., 2015; Zanoveli et al., 2015), which can explain the higher incidence of depression among diabetic (DBT) patients (Anderson et al., 2001, Smith et al., 2013; Nicolau et al., 2013). Depression has been related to a general worsening in the health of DBT patients, increasing the morbidity and mortality (Nefs et al., 2016). It has been proposed that this comorbidity may be the result of changing in lifestyle (dietary restriction, chronic daily treatment, increased in financial expenses and in frequency of hospitalization) and/or of physiological changing due to the diabetic condition (Wredling et al., 1992; Zanoveli et al., 2015).

Many proposals have been made to try to understand the pathophysiological relationship between depression and diabetes. For example, it has been reported in animal models of diabetes several brain alterations, such as disturbed neurotransmission (Bellush et al., 1991; Gupta et al., 2014; Prabhakar et al., 2015), impaired synaptic plasticity, reduction of adult neurogenesis in the dentate gyrus, increased oxidative stress in brain areas related to depression such as hippocampus (HIP) and prefrontal cortex (PFC) (Beauquis et al., 2010; Wayhs et al., 2010; Naudi et al., 2012; de Morais et al., 2014). Indeed, the cause of depression associated with diabetes is still not conclusive, leading to the proposal of ineffective treatments. To worsen, the antidepressant chronic treatment may induce significant effects over diabetic condition, including disturbed patient's blood glucose levels (Zanoveli et al., 2015). In that sense, it is important to note that in recent years a system has gained attention, the endocannabinoid system, which plays a regulatory role in several brain functions and seems to be a potential therapeutic target for the diabetes (Di Marzo et al., 2011) and depression treatments (Hill and Gorzalka, 2005; Mclaughlin et al., 2007; Booz, 2011; Micale et al., 2013, 2015, Gatta-Cherifi and Cota, 2015).

The endocannabinoid system includes the endogenous ligand araquidoniletanolamide (anandamide; AEA) that acts as atypical neurotransmitter being formed postsynaptically on demand by excitatory action. Consequently, this endogenous compound is released into the synaptic cleft acting in a retrograde manner by activating CB1 or CB2 receptors located pre-synaptically and thus inhibiting the release of different neurotransmitters, such as noradrenaline (NA) and serotonin (5-HT) (Devane et al., 1992; Fonseca et al., 2013). Therefore, the endocannabinoid system seems to act protecting the brain of being overwhelmed by excessive excitatory or inhibitory activity. The endocannabinoid system dysfunctions, such as extreme excitation or inhibition, may occur and lead to neuropsychological states, such as mania or hyperexcitability, at one extreme and depression, anhedonia and apathy on the other. Thus, a decrease of the endocannabinoid system activity could explain the anhedonia, anxiety, and also a decrease in the serotonergic activity that often accompanies depression (Wilson et al., 2001; Ashton and Moore, 2011).

Besides a possible role of the endocannabinoid system in the pathophysiology of neuropathologies commonly reported in DBT patients, this system seems to be particularly involved in the diabetic pathogenesis itself. In this sense, abnormalities in the endocannabinoid system have been reported in both patients and animal models of type 1 and type 2 diabetes (for review see Di Marzo et al. (2011)). Interestingly, patients with type 2 diabetes exhibit increased activity of the endocannabinoid system in visceral fat and increased plasma concentrations of AEA and 2-arachidonoylglycerol (2-AG; Matias et al., 2006). Besides, studies show that monoacylglycerol lipase (MGL) activity is increased in adipocytes from animal models of diabetes (Cable et al., 2014). Considering the type 1 diabetes, current research showed that endocannabinoids have immunosuppressive properties, such as leukocyte proliferation inhibition, reduction of pro-inflammatory cytokines and T cells apoptosis induction (for review see Katchan et al. (2016)). Given the role of the endocannabinoid system also in glucose homeostasis, in the food intake and energy balance (for review see Cristino et al. (2014)), cannabinoid receptor antagonists, mainly CB1 receptor antagonist, have been shown to be effective in weight and hyperinsulinemia reduction (Matias et al., 2006), in beta cells proliferation (Kim et al., 2011) and dyslipidaemia and blood pressure reduction (for review see Scheen and Paquot (2009)) while CB2 receptor agonist seems to be a potential therapeutic alternative to diabetic nephropathy (Zoja et al., 2016).

It is known that brain areas such as HIP and PFC, two brain areas extremely involved in the neurobiology of depression, together with the amygdala and ventral striatum present high densities of CB1 receptors (Herkenham et al., 1991; Mato and Pazos, 2004; Kano et al., 2009).