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Research Paper

Cortical adrenoceptor expression, function and adaptation under conditions of cannabinoid receptor deletion

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ABSTRACT

A neurochemical target at which cannabinoids interact to have global effects on behavior is brain noradrenergic circuitry. Acute and repeated administration of a cannabinoid receptor synthetic agonist is capable of increasing multiple indices of noradrenergic activity. This includes cannabinoid-induced 1) increases in norepinephrine (NE) release in the medial prefrontal cortex (mPFC); 2) desensitization of cortical α2-adrenoceptor-mediated effects; 3) activation of c-Fos in brainstem locus coeruleus (LC) noradrenergic neurons; and 4) increases in anxiety-like behaviors. In the present study, we sought to examine adaptations in adrenoceptor expression and function under conditions of cannabinoid receptor type 1 (CB1r) deletion using knockout (KO) mice and compare these to wild type (WT) controls. Electrophysiological analysis of α2-adrenoceptor-mediated responses in mPFC slices in WT mice showed a clonidine-induced α2-adrenoceptor-mediated increase in mPFC cell excitability coupled with an increase in input resistance. In contrast, CB1r KO mice showed an α2-adrenoceptor-mediated decrease in mPFC cell excitability. We then examined protein expression levels of α2- and β1-adrenoceptor subtypes in the mPFC as well as TH expression in the locus coeruleus (LC) of mice deficient in CB1r. Both α2- and β1-adrenoceptors exhibited a significant decrease in expression levels in CB1r KO mice when compared to WT in the mPFC, while a significant increase in TH was observed in the LC. To better define whether the same cortical neurons express α2A-adrenoceptor and CB1r in mPFC, we utilized high-resolution immunoelectron microscopy. We localized α2A-adrenoceptors in a knock-in mouse that expressed a hemoagglutinin (HA) tag downstream of the α2A-adrenoceptor promoter. Although the α2A-adrenoceptor was often identified pre-synaptically, we observed co-localization of CB1r with α2-adrenoceptors post-synaptically in the same mPFC neurons. Finally, using receptor binding, we confirmed prior results showing that α2A-adrenoceptor is unchanged in mPFC following acute or chronic exposure to the synthetic cannabinoid receptor agonist, WIN 55,212-2, but is increased, following chronic treatment followed by a period of abstinence. Taken together, these data provide convergent lines of evidence indicating cannabinoid regulation of the cortical adrenergic system.

1. Introduction

Norepinephrine (NE), a biogenic amine integral to the stress-response system and regulation of higher cognitive functions (Degenhardt et al., 2001; Arendt and Munk-Jorgensen, 2004; Pattij et al., 2008) is modulated by the endocannabinoid (eCB) system (Page et al., 2007; Page et al., 2008; Reyes et al., 2009; Carvalho et al., 2010b; Wyrofsky et al., 2015). Emerging lines of evidence indicate that the neurobehavioral consequences of exogenous cannabinoid exposure involve, in part, cannabinoid-induced cellular and molecular changes in adrenergic neurons. For example, pre-treatment of human subjects with the beta-adrenergic antagonist, propranolol, has been shown to prevent the acute effects of cannabis-induced impairment of learning (Sulkowski and Vachon, 1977). Likewise, using an animal model, pre-test administration of the α1-adrenergic agonist, phenylephrine, reversed post-training cannabinoid-induced retrieval impairment (Moshfegh et al., 2011). The efficacy of nabilone, a cannabinoid receptor agonist, in the treatment of post-traumatic stress disorder (PTSD) symptoms is attributed to actions of cannabinoids on NE circuitry (Reyes et al., 2009; Villanueva et al., 2009; Carvalho et al., 2010a; Carvalho et al., 2010b). Finally, mice deficient in CB1 receptor exhibit anxiogenic-like responses in various behavioral paradigms including

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the elevated plus-maze, the open field test and the light-dark box (Haller et al., 2002; Maccarrone et al., 2002; Martin et al., 2002; Uriguen et al., 2004; Thiemann et al., 2009). These mice also exhibited depressive-like phenotypes (Viveros et al., 2005; Valverde and Torrens, 2012), cognitive impairments including memory and learning deficits (Martin et al., 2002; Varvel and Lichtman, 2002; Bilkei-Gorzo et al., 2005; Madronal et al., 2012) as well as impairment in the extinction of aversive memories (Marsicano et al., 2002) with no changes in locomotor function (Haller et al., 2002) suggesting an effect on biogenic amine circuitry.

Cannabinoid receptor agonists have been shown to both increase, and decrease, indices of brain noradrenergic activity. Activation of presynaptic CB1 receptor on terminals of sympathetic axons innervating blood vessels has been shown to reduce the release of NE (Ishac et al., 1996; Pfitzer et al., 2005). Incubation of synaptosomes with low concentrations of tetrahydrocannabinol (THC) results in reductions in NE release (Poddar and Dewey, 1980) while systemic administration of rimonabant (a CB1 receptor antagonist) increases NE in the anterior hypothalamus and medial prefrontal cortex (mPFC) not the nucleus accumbens (Tzavara et al., 2001; Tzavara et al., 2003). In addition to inhibition of NE release, several lines of evidence support cannabinoid-induced increases in NE release (Rodriguez de Fonseca et al., 1991; Molderings et al., 2002; Page et al., 2007). Acute systemic (Oropeza et al., 2005) or local (Page et al., 2008) administration of the synthetic cannabinoid receptor agonist, WIN 55,212-2, or administration of a FAAH inhibitor (Gobbi et al., 2005), increases NE efflux in the rat frontal cortex. Acute WIN 55,212-2 exposure stimulates c-Fos expression in noradrenergic neurons of the locus coeruleus (LC) (Oropeza et al., 2005; Page et al., 2008), enhances *N*-methyl-D-aspartate-induced firing of LC neurons (Mendiguren and Pineda, 2004) and increases NE synthesis (Moranta et al., 2009) and release in terminal regions (Oropeza et al., 2005). CB1 receptor have been localized to noradrenergic axon terminals in the mPFC (Oropeza et al., 2007) supporting the hypothesis that NE and eCBs can regulate each other's function.

We have also described functional interactions between CB1 receptor and adrenoceptor systems in the mPFC. Whole cell patch clamp recordings of layer V/VI cortical pyramidal neurons in rats revealed that clonidine-induced α2-adrenoceptor-mediated elevations in cortical pyramidal cell excitability are significantly decreased following pre-treatment with the synthetic CB1 receptor agonist, WIN 55,212-2, suggesting cannabinoid stimulation of NE release and desensitization of α2-adrenoceptors (Reyes et al., 2012). The receptor interaction was both action potential and $GABA_A$ receptor-independent as the desensitization occurred similarly in the presence or absence of tetrodotoxin or the $GABA_A$ receptor antagonist bicuculline indicating that CB1-α2-AR interactions are likely direct rather than mediated by synaptic afferents. We also showed that α2A-adrenoceptors-immunoreactivity is distributed in axon terminals, somata and dendrites in the mPFC using immunoelectron microscopy (Cathel et al., 2014), consistent with other reports (Aoki et al., 1994; Venkatesan et al., 1996; Aoki et al., 1998; Aoki et al., 2000). Systemic administration of WIN 55,212-2, tetrahydrocannabinol (Δ^9 THC) and CP 55940 increases the spontaneous firing rate of LC neurons in a dose dependent manner (Mendiguren and Pineda, 2006; Muntoni et al., 2006). These effects were prevented by pretreatment with the cannabinoid receptor (CB1 receptor) antagonist, SR 141716 (Oropeza et al., 2005; Mendiguren and Pineda, 2004) supporting the involvement of CB1 receptors.

Adaptations occur following chronic exposure to WIN 55212-2 and withdrawal. For example, repeated administration of WIN 55212-2 increases TH protein expression in the LC and this is accompanied by potentiated NE efflux in response to an acute injection of WIN 55212-2 without a change in baseline NE efflux (Page et al., 2007). Chronic WIN 55,212-2 administration produces an anxiogenic-like response that reverts to pre-drug levels following a period of abstinence (Page et al., 2007). Chronic WIN 55212-2 treatment completely abolishes the ability of clonidine to induce an increase in excitability of PFC neurons (Reyes et al., 2012) and reduces the binding site density of ß1-adrenoceptors in neocortex (Hillard and Bloom, 1982; Reyes et al., 2009), and both α2- and ß1-adrenoceptors in the accumbens (Carvalho et al., 2010b). Meanwhile withdrawal following chronic WIN 55212-2 exposure alters ß1-adrenoceptors in the mPFC (Reyes et al., 2009). Taken together, these data indicate that sustained CB1 receptor activation results in a sustained increase in NE release which induces down-regulation of adrenergic receptors.

However, several gaps in knowledge remain that we addressed in the present study using a multidisciplinary approach. First, we measured electrophysiological properties of α2–adrenoceptor responses under conditions of CB1 receptor deletion using CB1 receptor-knockout (KO) mice and compared these to wild type (WT) controls. Next, we examined expression levels of α2- and β1-adrenoceptors in the mPFC in mice lacking the CB1 receptor as well as the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH), in the LC, which provides the sole source of NE to the mPFC (Halliday, 2004; Aston-Jones and Cohen, 2005; Aston-Jones et al., 2007). To better define sites of cortical cannabinoid-adrenoceptor interactions, we examined the ultrastructural localization of CB1 receptors with respect to neurons expressing α2-adrenoceptors in a knock-in mouse that expressed a hemoagglutinin (HA)-tag downstream of the α2-adrenoceptor promoter (Lu et al., 2009). Given the results showing significant adaptations in the adrenergic system under conditions of CB1r deletion, we next investigated α2A-adrenoceptor binding in the mPFC following acute and chronic WIN 55,212-2 and following abstinence from chronic WIN 55,212-2 exposure in rats. These data are important considering the increasing prevalence of clinical studies examining exogenous cannabinoid administration for the treatment of a variety of pathophysiological conditions. Furthermore, because the noradrenergic and eCB systems are both dynamically regulated by stress, where stress decreases anandamide and CB1 receptor levels while increasing 2-arachidonoyl glycerol levels (Morena et al., 2016), understanding the mechanism underlying the cannabinoid-adrenoceptor interactions in the mPFC may help unravel the mechanism underlying cannabinoid-induced impairments in attention and cognition.

2. Methods

The procedures employed in the present study conformed to Drexel University Institutional Animal Care and Use Committee, *National Institute of Health's Guide for the Care and Use of Laboratory Animals* (1996), the Health Research Extension Act (1985) and the PHS Policy on Humane Care and Use of Laboratory Animals (1986). All efforts were made to utilize only the minimum number of animals necessary to produce reliable scientific data, and experiments were designed to minimize any animal distress.

2.1. Specificity of CB1 receptor antibody

Three male CB1r KO mice and three WT controls mice (9–12 weeks old) were deeply anesthetized with sodium pentobarbital (40 mg/kg) and perfused transcardially through the ascending aorta with heparinized saline followed with 25 ml of 4% formaldehyde in 0.1 M phosphate buffer (PB; pH 7.4). Immediately following perfusion/fixation, the brains were removed and postfixed for 30 min. Brains were sectioned in the coronal plane at a setting of 40 μm using a Vibratome (Technical Product International, St. Louis, MO, USA) through the forebrain and hippocampus, and collected into 0.1 M PB. Sections through the rostrocaudal extent of mPFC and hippocampus were processed for light microscopic detection of CB1 receptor in the mPFC. Tissue sections were incubated in rabbit anti-CB1 receptor at 1:1000 in 0.1% bovine serum albumin (BSA), 0.25% Triton X-100 and 0.1 M tris buffered saline (TBS; pH 7.6) for 15–18 h at room temperature. The following day, tissue sections were rinsed three times in 0.1 M TBS and incubated in biotinylated donkey anti-rabbit (1:400; Vector Laboratories, Burlingame,