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The dual FAAH/MAGL inhibitor JZL195 has enhanced effects on endocannabinoid transmission and motor behavior in rats as compared to those of the MAGL inhibitor JZL184

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ABSTRACT

The biological actions of the endocannabinoids anandamide and 2-arachidonoyl glycerol (2-AG) are terminated by enzymatic hydrolysis of these lipids *via* fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively. While several selective FAAH inhibitors have been developed and characterized *in vitro* and *in vivo*, none of the initial MAGL blockers have shown adequate potency and specificity for *in vivo* applications. More recently, a selective MAGL inhibitor, JZL184, has been shown to produce a long-lasting elevation of brain 2-AG, as well as cannabinoid-like behavioral responses in mice. However, its effectiveness in rats remains controversial. Indeed, although JZL184 can elicit behavioral responses that are mediated, at least in part, *via* activation of cannabinoid CB1 receptors, several reports indicate that this compound does not alter 2-AG levels in this species. In this study we compared the behavioral and neurochemical effects of JZL 184 with those of the dual FAAH/MAGL inhibitor JZL195, and showed that systemic administration of the former can selectively elevate brain 2-AG in rats and produce motor suppression through a CB_1-independent mechanism. These findings indicate that, despite its lower potency against rat MAGL, JZL184 can be used to enhance 2-AG transmission and elicit behavioral responses in rodents.

1. Introduction

The endocannabinoids are neuromodulatory signaling molecules implicated in a large range of biological processes including cognition, analgesia, stress, anxiety, neuroprotection, and motor coordination, to name a few (Di Marzo, 2008; Giuffrida et al., 1999; Hohmann et al., 2005; Marsicano et al., 2002; Piomelli, 2008). The most studied endocannabinoids, N-arachidonoyl ethanolamine (anandamide, AEA) and 2-arachidonoyl glycerol (2-AG), are primarily synthesized on demand following an increase of intracellular calcium or neuronal depolarization (Piomelli, 2005). Endocannabinoids activate two G-protein coupled cannabinoid receptors (CB_1 and CB_2), but they can also target other non-CB_1/CB_2 receptors, showing a complex pharmacological profile (Di Marzo and De Petrocellis, 2010; O'Sullivan, 2007).

The biological actions of AEA and 2-AG are terminated by a two-step process including their reuptake into neuronal and glial cells followed by intracellular hydrolysis. While the existence of an active reuptake remains controversial (Fegley et al., 2004; Fu et al., 2012; McFarland et al., 2004), the catabolic enzymes responsible for AEA and 2-AG degradation — fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL), respectively — have been extensively characterized (Cravatt et al., 1996; Dinh et al., 2002). In addition to AEA, FAAH hydrolyzes monoacyl glycerols *in vitro* (Goparaju et al., 1998) and possibly participates in the inactivation of 2-AG (Di Marzo et al., 1998) together with the serine hydrolases ABHD6 and ABHD12 (Marrs et al., 2010; Savinainen et al., 2012). Nevertheless, several lines of evidence indicate that MAGL is the primary enzyme responsible for 2-AG degradation (Blankman et al., 2007; Dinh et al., 2004; Long et al., 2009a).

These findings have led to the development of selective inhibitors that are used as pharmacological tools to manipulate AEA and 2-AG signaling independently and to study their possible interactions (King et al., 2007; Saario et al., 2005). None of the initial MAGL inhibitors, however, showed adequate potency and specificity for *in vivo* applications (Long et al., 2009c). More recently, Long et al. (2009c) developed a dual FAAH/MAGL inhibitor, named JZL195, and a selective MAGL blocker, JZL184, which caused a rapid and long-lasting elevation of 2-AG in mouse brain without affecting AEA content (Long et al., 2009a). The same lab also developed new MAGL inhibitors — *i.e.* KML29 (Chang et al., 2012) and MJN110 (Niphakis et al., 2013) — showing enhanced activity and selectivity as compared to those of JZL184.

Microdialysis studies carried out in rat nucleus accumbens (NAc) have shown that JZL195 elevates both AEA and 2-AG, whereas JZL184

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has no effect on endocannabinoid output (Wiskerke et al., 2012). In agreement with its known lower activity against rat MAGL (Long et al., 2009b), other groups have shown unchanged 2-AG levels in rat CNS following systemic or local administration of JZL184 (Kerr et al., 2013; Woodhams et al., 2012). Despite the lack of neurochemical effects, JZL-184 produces behavioral effects mediated *via* activation of CB_1 receptors (Long et al., 2009a; Woodhams et al., 2012). Thus, these observations indicate that JZL184 effects in rats might be dose-dependent and/or area-specific, leading to 2-AG elevation only in those brain regions where this lipid is produced on demand.

To address this question, we compared the effects of JZL195 and JZL184 in rats, focusing on locomotor activity — a behavior typically affected by cannabinoids — and on endocannabinoid transmission in cortical-, striatal- and hippocampal-like brain areas.

2. Materials and methods

2.1. Drugs

JZL184 and JZL195 were a gift from Dr. J. Long (Scripps Research Institute, San Diego); SR141716A (synthesized at the Research Technology Branch of the National Institute on Drug Abuse, Rockville, MD) was a gift from Dr. McMahon at the University of Texas Health Science Center at San Antonio (UTHSCSA); Tween-80 and polyethylene glycol (PEG) were purchased from Sigma/RBI (St Louis, MO); AM251 was from Tocris (Ellisville, MO); methanol, chloroform, water, hexane from Honewell/Burdick & Jackson (Muskegon, MI); [$^2H_5$]-2-arachidonoyl glycerol (2-AG-*d5*) from Cayman Chemicals (Ann Arbor, MI); BSTFA from Supelco (Bellefonte, PA) and saline solution from Hospira, Inc. (Lake Forest, IL). [$^2H_4$]-anandamide (AEA-*d4*) and [$^2H_4$]-oleylethanolamide (OEA-*d4*) were synthesized in the lab as previously described (Hardison et al., 2006).

2.2. Animals

All experiments were carried out according to the NIH Guide for the Care and Use of Laboratory Animals and approved by the IACUC of UTHSCSA. Male Wistar rats (200–225 g; Charles River Laboratories, USA) were housed at 22 ± 1 °C, under a 12-h light/dark cycle with food and water available *ad libitum*. Upon arrival, the animals were habituated to the housing conditions for 1 week before the experimental testing, which was carried out during the light period of the cycle.

2.3. Drug treatments and behavioral tests

The animals were transferred into the experimental room in their own home cages and then placed in the ActiMot Activity Measuring System (version 6.07, TSE Systems GmbH, Bad Homburg, Germany) 1 h after receiving an acute intraperitoneal (i.p.) injection of vehicle (Tween-80/PEG/saline, 10/10/80, 1 ml/kg), or either JZL195 or JZL184

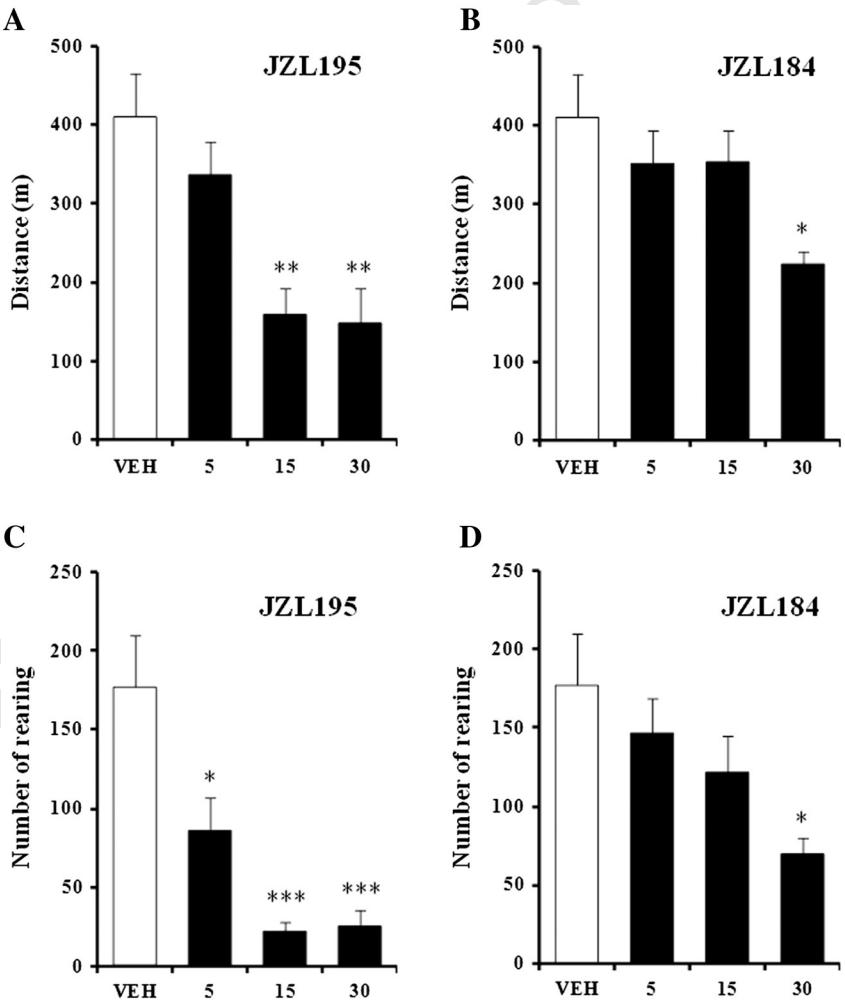

Fig. 1. Dose response of the effects of systemic (i.p.) administration of JZL195 (A, C) and JZL184 (B, D) on horizontal (traveled distance) and vertical (number of rearings) activities in a novel environment. Values are expressed as mean ± S.E.M. (n = 6–7/group). VEH, vehicle (empty bars); escalating doses of drugs (filled bars). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to vehicle control.

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