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COMMENTARY

Inhibitors of monoacylglycerol lipase as novel analgesics

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2-Arachidonoylglycerol (2-AG) is an endogenous cannabinoid (endocannabinoid) lipid whose functions remain poorly understood. Guindon and colleagues report the novel finding that exogenous application of 2-AG induces peripheral antinociceptive effects that are mediated, at least in part, by actions at peripheral cannabinoid CB_2 receptors. URB602, a recently described inhibitor of monoacylglycerol lipase, an enzyme that catalyzes 2-AG hydrolysis *in vivo*, also induced peripheral antinociceptive effects and enhanced the actions of 2-AG. Peripheral analgesic mechanisms represent promising therapeutic targets for suppressing pain in the absence of unwanted central nervous system side-effects (e.g. psychoactivity) associated with activation of central CB_1 receptors. The therapeutic potential of inhibitors of 2-AG deactivation for the treatment of inflammatory pain is discussed.
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Abbreviations: 2-AG, 2-arachidonoylglycerol; CNS, Central nervous system; DGL, diacylglycerol lipase; FAAH, fatty-acid amide hydrolase; MGL, monoacylglycerol lipase

Activation of cannabinoid CB_1 receptors – receptors that share the same target as Δ^9-tetrahydrocannabinol, the active ingredient in cannabis – suppresses nociceptive transmission and pain behavior in rodent subjects (for review see Walker and Hohmann (2005)). Nonetheless, psychoactive effects also accompany direct activation of CB_1 receptors and severely constrain the therapeutic potential of direct acting cannabinoid agonists. Recently, inhibitors of the deactivation of endogenous cannabinoid lipids (endocannabinoids) such as anandamide and 2-arachidonoylglycerol (2-AG) have been described. Inhibitors of fatty-acid amide hydrolase (FAAH), an enzyme which catalyzes anandamide hydrolysis (Cravatt *et al*., 1996), have been evaluated for their therapeutic potential for suppressing pain, anxiety and stress-related responses in preclinical studies (Kathuria *et al*., 2003; Hohmann *et al*., 2005). FAAH inhibitors produce their pharmacological effects by increasing levels of anandamide (and other fatty acid amides), thereby indirectly activating cannabinoid receptors, and produce a more circumscribed spectrum of biological effects compared to direct acting CB_1 agonists (Cravatt and Lichtman, 2003; Piomelli, 2005). The development of selective FAAH inhibitors, together with the generation of mutant mice lacking the FAAH gene, has considerably broadened our understanding of the physiological roles of anandamide in the nervous system. By contrast, the functional roles of 2-AG in the nervous system are only beginning to be explored.

Although FAAH has been shown to catalyze hydrolysis of 2-AG *in vitro* (Goparaju *et al*., 1999), a distinct enzyme, monoacylglycerol lipase (MGL) plays the predominant role in catalyzing 2-AG hydrolysis *in vivo* (Dinh *et al*., 2002, 2004; Hohmann *et al*., 2005). Recently, we have described the development of the first selective pharmacological inhibitor of MGL, URB602 (Hohmann *et al*., 2005). URB602 has been shown to increase levels of 2-AG without altering levels of anandamide both *in vitro* and *in vivo* (Hohmann *et al*., 2005), consistent with the selectivity of this compound in inhibiting cytosolic MGL relative to membrane FAAH activity. This compound induced a CB_1-mediated enhancement in endocannabinoid-mediated stress-induced analgesia following local administration into either the periaqueductal gray (PAG) or lumbar dorsal horn (Hohmann *et al*., 2005; Suplita *et al*., 2006). The URB602-induced enhancement of stress antinociception was associated with a profound increase in levels of 2-AG, but not anandamide, in the PAG. These studies support the existence of a physiological role for 2-AG in suppressing pain and raise the possibility that novel therapeutic interventions may inhibit MGL to suppress pain by increasing the bioavailability of endogenous 2-AG. However, the efficacy of MGL inhibition in suppressing chronic pain states has yet to be evaluated.

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The present report by Guindon and co-workers (Guindon *et al*., 2006) demonstrates that exogenous 2-AG induces peripheral antinociceptive effects in a tissue injury model of persistent pain, the formalin test. In this study, 2-AG, applied locally in the rat hind paw, suppressed formalin-evoked pain behavior during both the early and the late phase of formalin pain. 2-AG may induce peripheral antinociceptive effects by specifically suppressing formalin-evoked primary afferent activation and subsequent central nervous system (CNS) sensitization. More work is necessary to demonstrate that endogenous 2-AG is mobilized in the periphery under physiological conditions to suppress inflammatory nociception. In support of this hypothesis, the MGL inhibitor URB602, administered locally to the paw, induced antinociception in this model and enhanced the antinociceptive effects of exogenously applied 2-AG, thereby demonstrating the efficacy of an MGL inhibitor in suppressing inflammatory nociception. The observed antinociceptive effects were not secondary to a suppression of formalin-evoked edema. Mediation by cannabinoid receptors was demonstrated by the observation that selective antagonists for either CB_1 (AM251) or CB_2 (AM630) receptors blocked URB602-induced antinociception. By contrast, exogenous 2-AG produced peripheral antinociceptive effects that were blocked by the CB_2 but not the CB_1 antagonist. It is possible that inhibition of MGL with URB602 elevated levels of other monoglycerides, which do not activate cannabinoid receptors in addition to elevating levels of 2-AG. Non-endocannabinoid monoglycerides would be expected to compete with endogenous 2-AG for MGL-mediated hydrolysis; such 'entourage effects' (Ben-Shabat *et al*., 1998) could effectively enhance actions of endocannabinoids at CB_1 receptors, producing a peripheral antinociceptive effect susceptible to blockade by AM251. Alternatively, a CB_1-mediated blockade of locally administered 2-AG may not have been detected with the relatively modest sample sizes employed. More work is necessary to determine whether the doses of URB602 that suppressed formalin-evoked pain behavior also increased accumulation of endogenous 2-AG under the conditions of testing. It remains to be determined whether URB602 produces its pharmacological effects *in vivo* by targeting the cloned form of MGL rather than another as yet uncharacterized 2-AG hydrolyzing enzyme. Guindon and co-workers demonstrate that local administration of either the CB_1 or CB_2 antagonist alone failed to induce hyperalgesia, suggesting that endocannabinoids do not act tonically in the periphery to dampen sensitivity to pain.

The endocannabinoid 2-AG is believed to be synthesized and released upon demand through a process initiated by activity-dependent or receptor-stimulated cleavage of membrane phospholipid precursors (for review see Piomelli, (2003)). In brain, endocannabinoids may function as retrograde messengers to modulate neuronal physiology at presynaptic CB_1 receptors (Wilson and Nicoll, 2001, 2002)

In vitro electrophysiological studies and studies in cell culture suggest that 2-AG may be formed by the consecutive activation of two distinct enzymes (Chevaleyre and Castillo, 2003; Jung *et al*., 2005). First, phospholipase C catalyzes formation of the 2-AG precursor 1,2-diacylglycerol (DAG) from membrane phosphoinositides. Second, diacylglycerol lipase (DGL) catalyzes hydrolysis of DAG to generate 2-AG. More work is necessary to demonstrate that these same mechanisms control 2-AG formation under physiological conditions and to identify the specific enzyme isoforms and cell types implicated in this process. A better understanding of the mechanisms responsible for 2-AG formation and deactivation may identify novel molecular targets that may be exploited for the treatment of pain in humans. Work by Guindon and co-workers suggest, for the first time, that an MGL inhibitor shows significant therapeutic potential for the treatment of inflammatory pain.

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Conflict of Interest

The author is a coinventor on a patent filed by Daniele Piomelli at the University of California at Irvine.

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