

## REVIEW

# Opioid and Endocannabinoid System in Orofacial Pain

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## Summary

Orofacial pain disorders are frequent in the general population and their pharmacological treatment is difficult and controversial. Therefore, the search for novel, safe and efficient analgesics is an important but still elusive goal for contemporary medicine. In the recent years, the antinociceptive potential of endocannabinoids and opioids has been emphasized. However, concerns for the safety of their use limit their clinical applications. The possibility of modulating the activity of endocannabinoids by regulation of their synthesis and/or degradation offers an innovative approach to the treatment of pain. A rat model of trigeminal pain, utilizing tongue jerks evoked by electrical tooth pulp stimulation during perfusion of the cerebral ventricles with various neurotransmitter solutions can be used in the pharmacological studies of nociception in the orofacial area. The aim of this review is to present the effects of pharmacological activity of opioids and endocannabinoids affecting the transmission of the sensory information from the orofacial area on the example of trigemino-hypoglossal reflex in rats.

## Key words

Orofacial pain • Opioid and cannabinoid receptors • Endocannabinoid system • Perfusion of cerebral ventricles • Trigemino-hypoglossal reflex

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## Introduction

The therapy of pain is currently one of the challenges of modern medicine. Orofacial pain disorders are frequent in the general population and their pharmacological treatment is difficult and controversial (Weiss *et al.* 2017). This is due in part to limited understanding of the pathophysiology of the pain disorders that arise from the structures innervated by the trigeminal nerve. The additional reasons include the paucity of animal models, which allow to evaluate the responsiveness of nociceptors the magnitude of nociceptive reaction by measuring baseline and stimulated release of neuropeptides during the activation of trigeminal afferents (Hargreaves 2011, Zubrzycka and Janecka 2002, Zubrzycka and Janecka 2011).

In recent years, an accumulating body of evidence has suggested the potential use of endocannabinoids and opioids as the modulators of orofacial pain transmission (Zubrzycka *et al.* 2011, Zubrzycka *et al.* 2017).

It has been demonstrated that the endogenous opioid system could be involved in cannabinoid antinociception and recent data have also provided evidence for a role of the endogenous cannabinoid system in opioid antinociception (Bushlin *et al.* 2010, Cichewicz 2004, Wilson-Poe *et al.* 2013).

Opioids and cannabinoids produce antinociception through separate (although possibly interrelated) mechanisms (Cichewicz 2004, Roberts *et al.* 2006, Tham *et al.* 2005).

They also exhibit overlapping neuroanatomical distribution and comparable functional neurobiological properties (Nawarro *et al.* 2001). Both receptor types are found in several brain regions known to participate in antinociception, including the periaqueductal central gray (PAG), raphe nuclei and central medial thalamic nuclei (Cichewicz 2004, Manzanares *et al.* 1999). Furthermore,  $\mu$ - and CB1 receptors have been shown to colocalize to the same neurons within the superficial dorsal horn of the spinal cord, the first site of synaptic contact for peripheral nociceptive afferents, raising the possibility of direct interactions between these receptor types on the same cell (Hohmann *et al.* 2005, Salio *et al.* 2001).

Interestingly, CB1 receptors can exist as homodimers and they may also form heterodimers or oligomers with one or more other classes of co-expressed GPCRs, in a manner that may lead to cross-talk between CB1 receptors and noncannabinoid sites (Pertwee 2006).

Interactions between endocannabinoids (ECs) and the opioid system during antinociception have been evaluated using cannabinoid receptor antagonists or inhibitors of enzymes that degrade ECs. Studies showing that treatment with cannabinoid receptor agonists can lead to opioid peptide release and that ECs are involved in the actions of opioid agonists confirm the synergistic interactions between these two receptor systems (Cichewicz 2004, Lau *et al.* 2014a).

It is generally accepted that tooth pulp stimulation in rats represents a valuable pain model and has been proposed as a model for trigeminal pain (Chapman *et al.* 1986).

The trigemino-hypoglossal reflex has been used in research on the properties of neuropeptides perfused through the cerebral ventricles. In this pain model, the amplitude of the evoked tongue jerks (ETJ) induced by tooth pulp stimulation is recorded. The magnitude of ETJ during perfusion of the cerebral ventricles with the neurotransmitters under investigation can be a measure of the effect of these compounds on the neural structures (Zubrzycka *et al.* 1997, Zubrzycki *et al.* 2015).

The pharmacology and molecular mechanisms of sensory transmission in the trigeminal system have not been fully identified so far, which is associated with the lack of effective treatment for pain syndromes originating from the orofacial area.

Therefore, the aim of this review is to present the effect of pharmacological activity of opioids and endocannabinoids affecting the transmission of sensory information from the orofacial area on the example of trigemino-hypoglossal reflex in rats.

## The opioid system

The opioid system consists of opioid receptors and their peptide ligands. Opioid receptors were discovered approximately 40 years ago and they belong to the G-protein coupled receptor (GPCR) family. The heterogeneity of opioid receptors and the existence of at least three main opioid receptor types, designated as  $\mu$ ,  $\delta$  and  $\kappa$  (or MOR, DOR and KOR, respectively) is widely accepted (Kieffer 1995). Opioid receptors and endogenous opioid peptides are most abundant in the central nervous system (CNS) but are also localized in many peripheral tissues of the mammalian organism (Le Merrer *et al.* 2009).

Opioid receptors can be activated by exogenous opiates, such as morphine and its derivatives, as well as by endogenous opioid peptides: enkephalins (ENK), dynorphins (DYN),  $\beta$ -endorphin ( $\beta$ -END) and endomorphins (EMs), which are the family of neurochemical messengers that exhibit high affinity for one or more of the opioid receptors (Przewlocki and Przewlocka 2001, Rokyta 2018).

Opiates exert their antinociceptive activity by activation of the  $\mu$ -opioid receptors. Therefore, the search for natural and synthetic ligands of this receptor has become an attractive goal. The discovery in 1997 of two endogenous ligands of the  $\mu$ -opioid receptor, endomorphin-1 (Tyr-Pro-Trp-Phe-NH<sub>2</sub>) and endomorphin-2 (Tyr-Pro-Phe-Phe-NH<sub>2</sub>), gave hope that they could be used as analgesics devoid of at least some of undesired side effects of morphine (Zadina *et al.* 1997).

However, the use of endomorphins (EMs) as analgesics is of little therapeutic value because these peptides are quickly degraded by enzymes and have a very limited ability to penetrate the blood-brain barrier (BBB) effectively due to their small size and hydrophilic nature. To overcome the limited access of exogenously administered EMs to the brain, numerous chemical modifications of their structure have been proposed. To date, the structure of endomorphins has been modified by the introduction of D-amino acids and unnatural amino acids with L and D configurations, or by cyclization of the linear sequences (Perlikowska *et al.* 2014).

## The endocannabinoid system

The endogenous cannabinoid system (ECS), which consists of three principal components, i.e. "classical" cannabinoids (CB1, CB2) and "non-classical" (TRPV1, GPR55, PPARs) receptors, endogenous ligands

(anandamide, AEA; 2-arachidonylglycerol, 2-AG) and enzymes, responsible for CB synthesis and degradation, has recently attracted much attention as a crucial site in pain pathophysiology (Fonseca *et al.* 2013). Both CB1 and CB2 receptors which are both GPCRs inhibit the activity of adenylyl cyclase (Kaminski 1996) and activate mitogen-activated protein (MAP) kinases (Bouaboula *et al.* 1995). Additionally, CB1 (Mackie and Hille 1992) and CB2 receptors (Atwood *et al.* 2012) inhibit the function of voltage gated calcium channels, while only CB1 receptors have been shown to modulate the activity of potassium channels (Hampson *et al.* 1995).

The CB1 receptors exist predominantly in the CNS, at central and peripheral nerve terminals, where they mediate inhibition of transmitter release (Fride 2002). CB2 receptors occur mainly in the peripheral locations in the cells and organs associated with the immune system and are involved in the control of inflammatory reactions, one of their roles being the modulation of cytokine release (Pandey *et al.* 2009). Thus, a common role of CB1 and CB2 receptors appears to be the modulation of ongoing release of chemical messengers from neurons or from immune cells, respectively.

Identification of cannabinoid receptors started the search for their endogenous ligands and changed the initial belief that cannabinoids are substances of exclusively plant origin. In 1992, Devane *et al.* described the isolation of a brain lipid, arachidonoyl ethanolamide, named AEA, which mimicked the behavioral actions of the plant-derived THC. AEA is a partial agonist of both, CB1 and CB2 receptors but has lower efficacy and affinity for CB2 than for CB1.

Mechoulam *et al.* (1995) and Sugiura *et al.* (1995), independently, identified a second endocannabinoid, 2-AG which turned out to be a full CB1 receptor agonist and has higher affinity and efficacy for CB1 and CB2 receptors than AEA. Both endocannabinoids are derivatives of arachidonic acid conjugated with either ethanolamine (AEA) or with glycerol (2-AG).

There are some other lipid substances isolated from the brain, which have affinity for CB1 or CB2 receptors. However, their affinity is much lower than that of classical endocannabinoids, AEA or 2-AG, and they are considered putative ligands of these receptors or cannabinoid-like compounds. They may probably act together with endocannabinoids, by inhibiting their inactivation. Among such cannabinoid-like compounds there is, more recently discovered, O-arachidonoyl ethanolamine (virodhamine) which is an ester of

arachidonic acid and ethanolamine, as opposed to the amide linkage found in AEA. It acts as an antagonist of CB1 receptor and agonist of CB2 receptor. Other important fatty acid amides related to endocannabinoids are oleoylethanolamide (OEA), an agonist of the CB1 receptor and palmitoylethanolamide (PEA), a selective CB2 receptor agonist (Di Marzo *et al.* 2001).

However, much attention has currently been given to the fatty acid amide hydrolase (FAAH) and its metabolites. FAAH is an intracellular enzyme, primarily located in the brain and the liver, as well as in peripheral organs (Ueda and Yamamoto 2000). It is commonly accepted that AEA is the main substrate for FAAH, but a growing body of evidence points that 2-AG, although not exclusively, is also deactivated by this hydrolase (Di Marzo *et al.* 2001). FAAH is also involved in the degradation of several other biolipids, including OEA and PEA, which may bind to both, “classical” and “non-classical” CB receptors. Therefore, it has been suggested that the modulators of the FAAH activity, as potential therapeutics, may provide finer tuning of the ECS function than the CB receptor ligands, also in terms of peripheral vs. central site-dependent side effects (Hanus and Mechoulam 2010).

Cannabinoids act synergistically with opioids and act as opioid-sparing agents, allowing lower doses and fewer side effects from chronic opioid therapy. The endocannabinoid system represents an ideal target because it is a key endogenous system in modulating pain-processing pathways (Mechoulam *et al.* 2014, Woodhams *et al.* 2017, Rokyta *et al.* 2018).

### **Common features of opioid and cannabinoid receptor systems**

There is considerable evidence describing similarities between the opioid receptor system and the cannabinoid receptor system. Activation of opioid or cannabinoid receptors can produce similar behavioral effects, including antinociception, suggesting a similar distribution and mechanism of action (Manzanares *et al.* 1999, Woodhams *et al.* 2017). Both receptor types are found in several brain regions known to participate in antinociception, including PAG, raphe nuclei and central-medial thalamic nuclei, suggesting that they may either act alone, or in tandem to produce antinociception (Hohmann *et al.* 1999). Furthermore,  $\mu$ - and CB1 receptors have been shown to colocalize to the same neurons within the superficial dorsal horn of the spinal cord (Hohmann *et al.* 1999, Salio *et al.* 2001), the first

site of synaptic contact for peripheral nociceptive afferents, raising the possibility of direct interactions between these receptor types within the same cell.

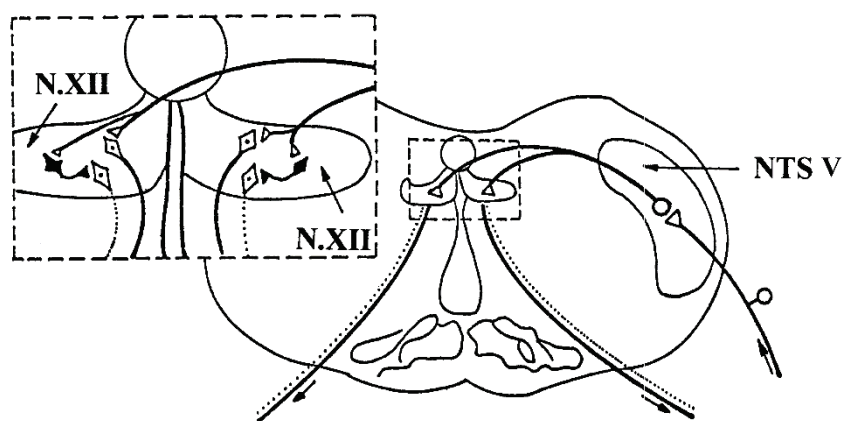
Opioid and cannabinoid receptors also share similar signal transduction properties. Both are GPCRs that 1) couple to G $\alpha$ i, blocking cAMP production, 2) activate MAP kinases through other second messenger systems, and 3) inhibit neurotransmitter release *via* inhibition of calcium channels and activation of potassium channels (Cichewicz 2004, Vigano *et al.* 2005). Both receptors types are generally found on presynaptic terminals, a location that is consistent with the inhibition of neurotransmitter release (Vigano *et al.* 2005, Rokyta 2018). These data raise the intriguing possibility that opioid and cannabinoid receptors function together within the same cell or neuronal circuit to

produce antinociception and that modulation of one receptor system may lead to alterations in the activity of the other.

### Neuronal organization of trigemino-hypoglossal reflex

The reflex that has been used for over a decade in research of the properties of neuropeptides perfused through the cerebral ventricles in rats is the evoked tongue jerk (ETJ) due to trigemino-hypoglossal reflex (Zubrzycka *et al.* 1997, Zubrzycki *et al.* 2017).

The trigemino-hypoglossal reflex arc extends below the floor of the IV ventricle between the sensory nucleus of the trigeminal nerve and the motor nucleus of the hypoglossal nerve (Fig. 1).



**Fig. 1.** A scheme of the trigemino-hypoglossal reflex arc. NTS V – n.V spinal tract nucleus; N.XII – hypoglossal nerve nucleus; light-colored triangle – the excitatory synapse, dark triangle – the inhibitory synapse.

The hypoglossal nerve nucleus is situated superficially under the floor of the fourth cerebral ventricle, which allows to suppose that chemical substances can penetrate to that nucleus from the IV ventricle lumen faster than to the motor trigeminal nerve nucleus, located deeper under the floor of the IV ventricle. The magnitude of the trigemino-hypoglossal reflex and the factor modulating the reflex depend on the branch of the trigeminal nerve, whose stimulation with electric impulses evokes contractions of the tongue muscles. The recorded amplitude of ETJ induced by tooth pulp stimulation is the measure of the effect of neuropeptides on the neural structures (Zubrzycka *et al.* 1997). The variability of effects of neuropeptides perfused through the cerebral ventricles of the trigemino-hypoglossal reflex can be explained by their different impact on the sensory neurons of n.V nuclei, interneurons and n.XII motoneurons. The stimulating or inhibitory effect is dependent on the binding site of the investigated compound with the membrane receptor, the time of its

release from the receptor, as well as its degradation time.

In the trigemino-hypoglossal reflex such as tongue jerk evoked by tooth pulp stimulation, the impulsion from n.V sensory nuclei was transferred to the ipsi- and contralateral hypoglossal nerve nucleus where it stimulated the motoneuron of the tongue-withdrawing muscles, and by excitation of the inhibitory interneuron caused inhibition of the tongue protrusion muscles motoneuron.

As indicated by the results of experiments involving perfusion of neuropeptides through the cerebral ventricles in rat obtained to date, the trigemino-hypoglossal reflex may serve as a good model for testing the effect of various neuropeptides on the brainstem centers.

### Neuropeptides in the cerebrospinal fluid

The cerebroventricular system may play the role of a humoral information channel in the CNS. The

cerebrospinal fluid (CSF), although it is not likely to contain biologically active endogenous neuropeptides, can be used as a vehicle to transport exogenous neuropeptides across the ependyma into the brain parenchyma following intracerebroventricular (i.c.v.) administration. Thus, due to the reversed concentration gradient following administration, exogenous neuropeptides in the CSF can potentially reach numerous, even very distant, locations within the brain (Bittencourt and Sawchenko 2000).

A neuropeptide which is administered i.c.v. is first diluted in the CSF, and then diffuses through the whole area of the ventricular and cerebellomedullary cistern walls into the ECF of the brain. From the ECF, it diffuses towards the target site, i.e. into the vicinity of structures responsible for the trigemino-hypoglossal reflex. Hence, the ultimate concentration of the neuropeptide following i.c.v. administration, which is effective in the specific structures, is significantly lower than the initial concentration.

Whether or not will the neuropeptide reach the brain, is largely dependent on its ability to cross the BBB, which depends on its lipophilic properties. Hydrophilic compounds penetrate that barrier less effectively. Opioids characterized by high lipophilicity permeate to the brain more easily (Abbott 2002).

Noxious stimuli may increase the release of endogenous opioid peptides, including  $\beta$ -endorphin ( $\beta$ -EP), in the midbrain PAG and in the hypothalamus, as well as their release to the CSF (Bach *et al.* 1995).

As demonstrated by clinical studies, the endorphin levels in the CSF may increase after stimulation with low-frequency alternating current. Stimulation of tooth pulp and nociceptive fibers results in a significant increase in the release of immunoreactive  $\beta$ -EP (Zubrzycka and Janecka 2011). The fibers containing  $\beta$ -EP are widely distributed in the brain and terminate in PAG, but their main projections extend along the cerebral ventricles, where they are in close contact with the ependyma of the third ventricle and the CSF-containing neurons (Veening and Barendregt 2010). Therefore, active transport of neuropeptides into the CSF might be effected *via* specialized ependymal cells – tanyocytes, extending from the third ventricle, where the sensory and motor centers of the trigemino-hypoglossal reflex are situated (Langlet *et al.* 2013). Also CB2 receptor activation by AM1241 causes a release of  $\beta$ -EP from keratinocytes, and the antinociceptive effect of AM1241 in rats is blocked by  $\mu$ -opioid receptor

antagonism and by  $\beta$ -EP antiserum (Ibrahim *et al.* 2005).

Some endocannabinoids have been shown to play a role in the regulation of BBB permeability. It has been demonstrated that AEA and OEA decrease BBB permeability *in vitro* in human cells in normal conditions (Hind *et al.* 2015). Used in *in vivo* and *in vitro* models, they increased BBB permeability following chronic head injury and multiple sclerosis decreased by the exogenous addition of AEA (Mestre *et al.* 2011) and 2-AG (Panikashvili *et al.* 2006).

In view of the above, changes of CSF composition should influence the somatic reflex whose centers are located in the vicinity of the cerebral ventricles.

### Effect of opioids on the trigemino-hypoglossal reflex

Endogenous opioid systems play an important role in the antinociceptive activity (Rokyta 2018). The involvement of opioid receptors in neuropeptide-induced modulation of the trigemino-hypoglossal reflex was confirmed by blocking of these receptors with naloxone (NAL) or a selective  $\mu$ -receptors antagonist –  $\beta$ -funaltrexamine ( $\beta$ -FNA) (Zubrzycka *et al.* 2005, Zubrzycka and Janecka 2008, Zubrzycka and Janecka 2011). Activation of PAG neurons is associated with the descending antinociceptive effect (Bourbia and Pertovaara 2018). Blocking with NAL the opioid receptors located in the centers adjacent to the cerebral ventricles disinhibited to a considerable extent the nociceptive trigemino-hypoglossal reflex due to PAG stimulation, which inhibits the reflex. The above evidences the role of opioid receptors in modulation of the reflex magnitude (Zubrzycka and Janecka 2001, Zubrzycka and Janecka 2011).

It has also been demonstrated that blocking opioid receptors in the centers adjacent to the cerebral ventricles with exogenous morphiceptin, ENK-Met,  $\beta$ -EP, EM-2 and based on the structure of EM-2 cyclic pentapeptide Tyr-c[D-Lys-Phe-Phe-Asp]NH<sub>2</sub>, (cEM-2), abolishes to a considerable extent the nociceptive ETJ reflex induced by tooth pulp stimulation in rats (reducing the magnitude of the reflex) (Zubrzycka and Janecka 2008, Zubrzycka and Janecka 2011, Zubrzycki *et al.* 2017).

Immunohistochemical studies have demonstrated that ENK-ergic neurons do not form pathways within the brain, but they occur in clusters as interneurons. Such

clusters have been observed both in the vicinity of the n.V spinal tract nucleus (NTS V) (Petrusz *et al.* 1985) and the n.XII nucleus (Connaughton *et al.* 1986). The presence of these neurons and ENK-ergic receptors on the cell membranes of n.XII nucleus motoneurons explains the inhibition of ETJ during perfusion of the cerebral ventricles with ENK.

EM-2 inhibits the amplitude of ETJ more intensively than morphiceptin and ENK-Met. The most potent antinociceptive properties have been demonstrated for cEM-2, which shows high affinity to the  $\mu$ - and CB1 receptors. Such an effect is associated with the fact that cyclization reduces the molecular conformational freedom, which is responsible for the activation of different receptors, increases metabolic stability and may increase lipophilicity, which often improves the blood-brain barrier BBB permeability for peptides (Zubrzycka and Janecka 2008, Zubrzycki *et al.* 2017).

### Effect of cannabinoids on the trigemino-hypoglossal reflex

Several lines of evidence have recently suggested that opioid and cannabinoid receptors can functionally interact in the CNS. These interactions may be direct, through receptor heteromerization, or indirect, through signaling cross-talk that includes agonist-mediated release and/or synthesis of endogenous ligands that can activate the downstream receptors (Bushlin *et al.* 2012).

Studies on the mechanisms underlying the analgesic effects of opioids and cannabinoids have largely focused on the GABA-ergic system within the PAG. Like opioids, cannabinoids are thought to produce analgesia by suppressing GABA-ergic inhibition of PAG output neurons, which project along a descending analgesic pathway (Lau *et al.* 2014b). ECs are described as ‘synaptic circuit breakers’ (Katona and Freund 2012), acting as retrograde neurotransmitters in the PAG and rostral ventromedial medulla, inhibiting GABA-ergic and glutamatergic transmission by preventing the release of transmitters from nerve terminals, *via* activation of CB1Rs (Vaughan *et al.* 1999).

The role of the endocannabinoid system in orofacial pain has also been investigated, demonstrating that the CB1 receptor ligand AEA exerts its analgesic effect *via* CB1 and  $\mu$ - receptors (Zubrzycki *et al.* 2017).

CB1 receptors are localized on fibres in the spinal trigeminal tract, found on large diameter

myelinated fibres and in the nucleus trigeminalis caudalis (Price *et al.* 2003). Therefore, it is also possible that AEA exerts a direct effect upon trigeminal neurons (Akerman *et al.* 2004) to cause inhibition of CGRP (calcitonine gene-related peptide) release from central terminals of primary afferent fibres and to reduce the nociceptive behaviour.

Blocking opioid and cannabinoid receptors with their antagonists ( $\beta$ -FNA and AM251) resulted in a significant decrease in the antinociceptive activity of AEA, confirming the involvement of opioid and cannabinoid systems in the modulation of the trigemino-hypoglossal reflex (Zubrzycki *et al.* 2017). As the elements involved in the trigemino-hypoglossal reflex arc are located in the vicinity of the cerebral ventricles, it can be assumed that  $\beta$ -FNA and AM251 injected into the cerebral ventricles permeated through the cerebroventricular lining and exerted their effects on the adjacent structures.

What seems surprising is that 2-AG had no significant effect on the amplitude of ETJ (Zubrzycki *et al.* 2017), as the PAG contains higher 2-AG than AEA levels under basal conditions (Maione *et al.* 2006, Petrosino *et al.* 2007). Furthermore, 2-AG has a higher efficacy as a full CB1 receptor agonist, despite the lower receptor affinity compared to AEA (Luk *et al.* 2004). This lower efficacy of 2-AG observed in our experiments might be due to the lower levels of 2-AG accessing presynaptic CB1 receptors and also to the fact that degradative enzyme for 2-AG is in closer proximity to the target CB1 receptor than AEA.

The literature data indicate that both AEA and 2-AG can independently regulate pain sensation (Lau *et al.* 2014b, Long *et al.* 2009a) and suggest that their individual activities are profoundly augmented upon simultaneous blockade of their respective degradative enzymes.

To verify this hypothesis in our pain model, we used the selective FAAH and MAGL (monoacylglycerol lipase) inhibitors URB597 and JZL184 respectively. URB597 shows high specificity for FAAH, significantly elevating the levels of AEA, OEA and PEA in the CNS and in peripheral tissues. PEA and OEA are capable of elevating the levels of AEA through substrate competition at FAAH (Alhouayek and Muccioli 2008, Kathuria *et al.* 2003).

In our experiments, we determined that inhibition of FAAH most likely resulted in an increase in AEA levels and enhanced antinociceptive effects. On the

other hand, inhibition of MAGL had no effect on the antinociceptive activity of 2-AG (Zubrzycki *et al.* 2017). Given the fact that FAAH and MAGL are localized in different regions of the neuron (e.g. FAAH postsynaptically, MAGL presynaptically) and regulate different ECs, the possibility exists that they may have different roles in regulating physiological functions, including nociception. It was shown that in acute pain models, effects of MAGL inhibition appear to be largely mediated by CB1 receptors (Long *et al.* 2009a), though a more prominent CB2-mediated component has been identified in inflammatory and neuropathic pain models (Guindon and Hohmann 2008), perhaps unsurprisingly since 2-AG is a full agonist at CB2 receptors while AEA is only a weak partial agonist of this receptor (Gonsiorek *et al.* 2000). Full inhibition of MAGL *via* JZL184 produces many cannabinoid-like behaviors (Long *et al.* 2009a), suggesting that this approach may share some of the unwanted side effects of cannabinoids. Furthermore, several studies have now revealed that sustained global elevation of 2-AG *via* genetic deletion of MAGL or persistent blockade of MAGL activity with enzyme inhibitors produces functional antagonism of the brain EC system (ECS), resulting in a profound down-regulation and desensitization of CB1 receptors in nociception-associated regions, and a loss of analgesic phenotype (Imperatore *et al.* 2015, Navia-Paldanius *et al.* 2015).

Long *et al.* (2009b) developed a new antagonist, JZL195, with dual inhibitory properties, simultaneously at MAGL and FAAH. Such inhibition might offer an attractive therapeutic approach, allowing to maintain analgesic efficacy at significantly lower doses while minimizing side effects associated with the direct action of cannabinoid agonists.

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Our results indicated that JZL195 enhanced the antinociceptive activity, most likely through the pharmacological elevation of AEA levels in the brain, and had greater antinociceptive efficacy than selective FAAH or MAGL inhibitors (Zubrzycki *et al.* 2017). Similar results were published by Long *et al.* (2009b), who showed that administration of JZL195 to mice resulted in reduction of brain FAAH and MAGL activities that correlated with near-complete inhibition of both AEA and 2-AG hydrolysis. These effects were accompanied by dramatic elevations of AEA and 2-AG brain levels that approximated the increases observed with selective FAAH (URB597) or MAGL (JZL184) inhibitors.

Thus, the possibility of modulating the activity of endocannabinoids by regulation of their synthesis and/or degradation offers an innovative therapeutic approach in many pathologic conditions (Hanus and Mechoulam 2010).

**In conclusion**, our data show that EC and opioid pathways are involved in the descending modulatory control of trigeminal nociceptive transmission from the brainstem, a mechanism hypothesized to contribute to the pathophysiology of orofacial pain. Additionally, these effects may imply that AEA, EM-2 and inhibitors of FAAH and FAAH/MAGL could be therapeutic in orofacial pain. Clinically, the data may offer the promise of an interesting avenue for therapeutic development.

## Conflict of Interest

There is no conflict of interest.

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