Published in final edited form as:
Life Sci. 2013 August 14; 93(0): 187–193. doi:10.1016/j.lfs.2013.06.004.

Sphingosine Lysolipids in the CNS: Endogenous Cannabinoid Antagonists or a Parallel Pain Modulatory System?

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Abstract

A significant number of patients experience chronic pain and the intractable side effects of currently prescribed pain medications. Recent evidence indicates important pain modulatory roles for two classes of G-protein-coupled receptors that are activated by endogenous lipid ligands, the endocannabinoid (eCB) and sphingosine-1-phosphate (S1P) receptors, which are widely expressed in both the immune and nervous systems. In the central nervous system (CNS), CB_1 cannabinoid and $S1P_1$ receptors are most abundantly expressed and exhibit overlapping anatomical distributions and similar signaling mechanisms. The eCB system has emerged as a potential target for treatment of chronic pain, but comparatively little is known about the roles of S1P in pain regulation. Both eCB and S1P systems modulate pain perception via the central and peripheral nervous systems. In most paradigms studied, the eCB system mainly inhibits pain perception. In contrast, S1P acting peripherally at $S1P_1$ and $S1P_3$ receptors can enhance sensitivity to various pain stimuli or elicit spontaneous pain. However, S1P acting at $S1P_1$ receptors and possibly other targets in the CNS can attenuate sensitivity to various pain stimuli. Interestingly, other endogenous sphingolipid derivatives might play a role in central pain sensitization. Moreover, these sphingolipids can also act as CB_1 cannabinoid receptor antagonists, but the physiological relevance of this interaction is unknown. Overall, both eCB and sphingolipid systems offer promising targets for the treatment of chronic pain. This review compares and contrasts the eCB and S1P systems with a focus on their roles in pain modulation, and considers possible points of interaction between these systems.

Sphingosine-1-phosphate (S1P) and the endocannabinoids (eCBs), their receptors and metabolic enzymes are all expressed in the central nervous system (CNS). S1P is the endogenous ligand for five S1P receptor (S1PR) types numbered 1 to 5 (Lee et al 1998, Rosen & Liao 2003). The eCBs, arachidonoylethanolamide (AEA; anandamide) and 2-arachidonoylglycerol (2-AG) primarily activate cannabinoid type 1 and 2 receptors (CB_1Rs and CB_2Rs) (Matsuda et al 1990, Munro et al 1993). Several lines of evidence indicate that

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the S1P and eCB systems interact in the CNS. S1PRs and CB_1Rs exhibit sequence homology, similarities in signaling and CNS localization, and produce overlapping, but independent, CNS-mediated effects (Pertwee et al 2010, Toman & Spiegel 2002, Welch et al 2012). This review will provide evidence that S1P and eCB systems in the CNS act in parallel, but have potential interactions, to modulate pain.

Endogenous Ligands

eCBs

AEA, a fatty acid amide (Devane et al 1992), and the lysolipid 2-AG (Sugiura et al 1995) are established eCB ligands. Other putative endogenous ligands include 2-arachidonoylglyceryl ether (2-AGE), virodhamine and N-arachidonoyl dopamine (NADA) (Walker et al 2002). Endocannabinoids are formed via calcium-dependent processes from membrane phospholipids (Alger & Kim 2011, Kano et al 2009). 2-AG is synthesized mainly via phospholipase C (PLC)-mediated formation of diacylglycerol (DAG) with subsequent cleavage of the *sn*1 fatty acid chain by DAG lipases (DAGL). A major pathway for AEA synthesis begins with transfer of arachidonic acid from phospholipids to the ethanolamine group of phosphatidylethanolamine via an N-acyltransferase reaction, followed by cleavage to AEA by N-acylphosphatidylethanolamine phospholipase D (NAPE-PLD). However, studies in NAPE-PLD knockout mice suggest additional routes of AEA synthesis in the CNS (Leung et al 2006). 2-AG and AEA are metabolized mainly by monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively, although other metabolic routes are possible (Ahn et al 2008, Kano et al 2009). Major eCB synthetic and degradative enzymes are expressed in the CNS (Ahn et al 2008, Kano et al 2009). Several components of the eCB system, including CB_1Rs, FAAH, DAGL and a putative AEA transporter, are found in lipid rafts, subdomains of plasma membranes that are rich in cholesterol and sphingolipids (Placzek et al 2008b). eCBs are generally synthesized postsynaptically and act retrogradely to inhibit GABA or glutamate release from presynaptic terminals, although this process is better defined for 2-AG than AEA (Alger & Kim 2011, Kano et al 2009). Depolarization-induced 2-AG formation is associated with short-term neuroplasticity termed depolarization-induced suppression of excitation (DSE; glutamate) or inhibition (DSI; GABA).

S1P

The enzymes that regulate S1P levels have also been identified (Le Stunff et al 2004). Ceramidases metabolize ceramide to sphingosine, which is then phosphorylated by sphingosine kinase (SphK) types 1 and 2 to produce S1P. Ceramide can be generated either via de novo synthesis in the endoplasmic reticulum or liberated from sphingomyelin in the plasma membrane by sphingomyelinases. CB_1R activation can increase de novo synthesis and sphingomyelin-liberated ceramide (Guzman et al 2001), suggesting that eCBs might regulate S1P production. Reversible inactivation of S1P occurs via dephosphorylation to sphingosine by S1P phosphohydrolases, whereas S1P lyase irreversibly cleaves S1P to phosphoethanolamine and hexadecenal. S1P (Edsall & Spiegel 1999) and sphingosine kinase activity (Blondeau et al 2007) are found throughout the brain. SphK immunoreactivity is found in dendrites in the cerebellar molecular layer (Terada et al 2004),

suggesting that S1P might be produced postsynaptically. However, the possibility that S1P is a retrograde neuromodulator like eCBs has not been investigated.

Exogenous Ligands

Cannabinoids

Δ^9-THC, the primary active constituent of marijuana, was isolated in the mid-1960s (Mechoulam & Gaoni 1967). The most frequently studied synthetic cannabinoid ligands include the CB_1/CB_2 agonists CP55,940 and WIN55,212-2 and the CB_1-selective antagonist rimonabant (SR141716A) (Howlett et al 2002, Rinaldi-Carmona et al 1994). CB_1-selective agonists include the AEA analogs ACPA and ACEA (Hillard et al 1999). CB_2-selective ligands include JWH-033 (agonist) and SR144528 (antagonist) (Huffman 1999, Rinaldi-Carmona et al 1998). Some synthetic cannabinoids that are added to abused herbal blends such as K2/Spice are potent and efficacious CB_1 agonists (Atwood et al 2010, Atwood et al 2011).

S1P Ligands

S1PR-selective ligands are comparatively limited. FTY720 or fingolimid (Gilenya™) (Brinkmann et al 2010), the first in class drug to target S1PRs, is FDA-approved to treat relapsing multiple sclerosis. FTY720 is an immunomodulatory prodrug that is structurally similar to sphingosine and is phosphorylated to the biologically active compound, phospho-FTY720 (FTY720-P), mainly by SphK 2. FTY720-P is an agonist or partial agonist for all S1PRs except $S1P_2R$. Its therapeutic effects are produced by $S1P_1Rs$ in the immune system and on glia (Chun & Brinkmann 2011). FTY720 efficiently crosses the blood brain barrier (Meno-Tetang et al 2006) and preferentially localizes in the CNS (Foster et al 2007).

$S1P_1R$-selective agonists (Lynch & Macdonald 2008, Rosen et al 2009) are being developed as therapeutics that might produce fewer side effects than FTY720 (Brinkmann & Lynch 2002). $S1P_1R$ agonists include SEW2871 (Sanna et al 2004), CYM-5442 (Gonzalez-Cabrera et al 2008) and KRP-203 (Shimizu et al 2005). The prodrug VPC01091 is an agonist or partial agonist at $S1P_{1,4,5}$ receptors, and an $S1P_3R$ antagonist, when phosphorylated in vivo by SphK 2 (Zhu et al 2007). $S1P_1R$ antagonists are also available. VPC44116 (Awad et al 2006) is a competitive antagonist at $S1P_1Rs$ that is ~10-fold less potent at $S1P_3Rs$ and is a partial agonist of $S1P_5Rs$ (Foss et al 2007). The $S1P_1$ antagonist W146 is slightly more $S1P_1$-selective than VPC44116 (Sanna et al 2006). Few selective ligands are available for other S1PR types. The limited availability of S1PR type-specific ligands, and limitations in CNS-permeability and solubility of some ligands, complicates the design and interpretation of *in vivo* studies.

Receptors

Both S1PRs and CB_1Rs are widely expressed in the CNS and in peripheral systems that contribute to pain regulation. $S1P_{1,2,3}$ receptors are distributed throughout the CNS and periphery, whereas $S1P_4R$ (lung, lymphoid and hematopoetic tissues) and $S1P_5R$ (brain white matter, spleen) expressions are restricted (Brinkmann 2007). Similarly, CB_1Rs predominate in the CNS whereas CB_2Rs are most highly expressed in the immune system

with limited CNS expression (Matsuda et al 1993, Munro et al 1993, Van Sickle et al 2005). S1PRs and CBRs belong to the superfamily of G-protein coupled receptors (GPCRs). S1P and dihydro-S1P bind to all S1PRs with high affinity (Rosen & Liao 2003). AEA and 2-AG bind to both CB_1 and CB_2 receptors (Howlett et al 2002), whereas the putative eCB 2-arachidonoylglycerol ether is CB_1R-selective (Hanus et al 2001). In addition, eCBs can bind to GPR55, transient receptor potential (TRP) channels and peroxisome proliferator-activated nuclear receptors (PPAR), which all are expressed in the CNS and can affect nociception (Pertwee et al 2010). Several lysolipid receptors, including those for S1P ($S1P_{1–5}$), lysophosphatidic acid ($LPA_{1–3}$) and eCBs ($CB_{1–2}$) are grouped into a related GPCR gene subfamily that also includes adenosine receptors and several orphan receptors (Pertwee et al 2010, Rosen & Liao 2003, Toman & Spiegel 2002). Novel cannabinoid-activated GPCRs are also found in the CNS (Breivogel et al 2001, Nguyen et al 2010) and might affect nociception, but have not been characterized.

S1PRs and CBRs couple to multiple G-proteins, which can regulate diverse signaling cascades. CB_1Rs activate primarily $G_{i/o}$ to inhibit adenylyl cyclase and calcium channels, and stimulate potassium channels, PLC, phosphoinositide-3-kinase (PI3K) and mitogen-activated protein (MAP) kinases (Howlett et al 2002). CB_1Rs can also activate G_s under certain conditions. S1P can function both intracellularly as a second messenger and extracellularly by activating S1PRs (Takabe et al 2008), which in turn regulate multiple signaling pathways (Brinkmann 2007, Rosen et al 2009). $S1P_{1–5}$ receptors activate $G_{i/o}$ and $S1P_{2–5}$ receptors also activate $G_{12/13}$. $S1P_{2,3}$ receptors also activate $G_{q/11}$, which increases intracellular calcium and activates protein kinase C. S1PRs can also activate MAPK or PI3K in certain cells. Thus, CB_1Rs and S1PRs modulate overlapping signaling pathways.

Both CB_1 and S1PRs are negatively regulated by prolonged agonist exposure. CB_1R desensitization and downregulation have been extensively characterized in cell models and the CNS (Sim-Selley 2003, Smith et al 2010). Studies in cell models have demonstrated that FTY720-P downregulates $S1P_1$Rs more effectively than S1P or SEW2871 (Gonzalez-Cabrera et al 2007, Oo et al 2007). These findings lead to the hypothesis that immunomodulatory effects of FTY720 could be due to “functional antagonism” of $S1P_1$Rs on lymphocytes, although an alternative hypothesis suggests that activation of $S1P_1$Rs on lymphoid epithelium contributes to immunomodulation (Rosen et al 2007, Schwab & Cyster 2007). While there is evidence to support both hypotheses, the fact that both CB_1Rs and $S1P_1$Rs are prone to agonist-induced downregulation suggests common regulatory mechanisms.

Receptor Localization in the CNS

Both CB_1Rs and S1PRs are highly expressed throughout the brain, but their colocalization has not been examined. CB_1Rs are most abundant in the basal ganglia, cerebellum, hippocampus and cerebral cortex (Glass et al 1997, Herkenham et al 1991, Tsou et al 1998). CB_1Rs are moderately expressed in thalamic and hypothalamic nuclei, brainstem and spinal cord. Immunohistochemistry revealed that CB_1Rs are localized predominantly on axons (Tsou et al 1998), consistent with their role in presynaptic regulation of neurotransmitter

release. CB_1Rs are also expressed by glia, with predominant functional expression in astrocytes (Stella 2010).

Localization of S1PR types in the CNS has been limited by the lack of type-selective probes. $S1P_{1,2,3}$ receptor mRNAs are expressed in neurons in monkey brain (Beer et al 2000). S1PRs are also expressed by astrocytes (Anelli et al 2005, Nishimura et al 2010, Rao et al 2003) and oligodendrocytes (Yu et al 2004). [^{35}S]GTPγS autoradiography showed high S1PR-mediated activity in the cerebral cortex, amygdala and molecular layer of the cerebellum, and moderate activity in the caudate-putamen, PAG, hippocampus, and hypothalamus (Sim-Selley et al 2009, Waeber & Chiu 1999). We used SEW2871, an $S1P_1$R-selective agonist, and VPC44116, an $S1P_{1>3}$ antagonist, to show that S1P1Rs represented 65–75% of S1P activity in the cerebral cortex, amygdala and cerebellum and ~40% of S1P activity in the caudate-putamen, hippocampus, hypothalamus and PAG (Sim-Selley et al 2009). SEW2871-stimulated [^{35}S]GTPγS binding was low in the corpus callosum, where $S1P_5$Rs likely predominate. Antagonist studies in the cingulate cortex and cerebellum confirmed that most activity in these regions resulted from $S1P_1$Rs.

Sphingosine and cannabinoid receptor interactions

We discovered a direct interaction between sphingolipid and eCB systems by demonstrating that sphingosine is a competitive antagonist of CB_1Rs (Paugh et al 2006). Both sphingosine and FTY720 competed with moderate affinity (Ki ~1 μM) for radioligand binding to CB_1Rs, but not CB_2Rs. In contrast, S1P competed for CB_1R binding with low affinity (>5 μM) and did not fully compete at concentrations up to 300 μM. These results suggest that only unphosphorylated sphingosine analogs have significant affinity for CB_1Rs, as supported by recent findings that dihydrosphingosine (sphinganine) and dimethylsphingosine (DMS), but not FTY720-phosphate and dihydro-S1P, compete for CB_1R binding (Selley et al 2011). Further experiments using [^{35}S]GTPγS binding revealed that both FTY720 and sphingosine are competitive antagonists of CB_1R-stimulated G-protein activity. Furthermore, both ligands inhibited cannabinoid agonist-stimulated CB_1R internalization and activation of MAP kinase (Paugh et al 2006). Conversely, S1P did not antagonize cannabinoid-stimulated G-protein activity in the same cell line, but modestly stimulated G-protein activation, which was likely due to endogenous S1PRs.

The physiological relevance of these findings is not yet clear. Nonetheless, eCBs and CB_1Rs are co-localized in lipid rafts (Barnett-Norris et al 2005, Placzek et al 2008a), and sphingosine and sphinganine would be present in these microdomains where they could potentially act as endogenous CB_1R antagonists. Moreover, S1P can regulate its own production from sphingosine through S1PR-induced increases in SphK activity (Meyer zu Heringdorf et al 2001). Therefore, S1PRs could indirectly modulate CB_1R function via local regulation of sphingosine levels. Furthermore, activation of glial CB_1Rs can stimulate production of the sphingosine precursor ceramide (Guzman et al 2001), providing a potential mechanism whereby CB_1R activation could regulate production of an endogenous CB_1R antagonist.

Lipid mediators as analgesic targets

Chronic pain is difficult to alleviate and many drugs currently prescribed have a number of disadvantages. Thus, the identification of new pharmacotherapeutic targets for chronic pain remains a priority. Cannabinoids produce analgesia in acute pain tests and alleviate several chronic painful conditions including inflammatory and neuropathic pain (Cravatt & Lichtman 2004, Guindon & Hohmann 2009). However, the clinical use of cannabinoids is limited by side effects, including psychoactive and behavioral effects. An alternative strategy is to inhibit eCB degradative enzymes to increase AEA and 2-AG levels, which can produce antinociception in the absence of overt negative side effects in animal models (Cravatt & Lichtman 2003, Long et al 2009).

The neurobiological mechanisms by which CB_1Rs modulate pain in the CNS have been extensively studied. Cannabinoid agonists produce antinociception via both spinal and supraspinal mechanisms (Lichtman & Martin 1991). Antinociception is produced by cannabinoid administration into the PAG (Martin et al 1995), rostral ventral medulla (Martin et al 1998) or thalamus (Martin et al 1996). CB_1Rs are also expressed in the superficial laminae (I and II) of the spinal cord (Farquhar-Smith et al 2000), dorsal root ganglion (Ahluwalia et al 2000) and peripheral nociceptors (Agarwal et al 2007). The eCBs are also implicated in antinociception because inhibitors of eCB degradation or transport produce analgesia that is blocked by antagonists or genetic deletion of CBRs (Cravatt & Lichtman 2004, Guindon & Hohmann 2009, Walker et al 2002).

Data from our laboratory and others suggest that the S1P system is a novel target for pain regulation (Welch et al 2012). We showed that intracerebroventricular (i.c.v.) S1P administration produced thermal antinociception, hypoactivity, catalepsy and hypothermia, but only antinociception was reversed by the $S1P_{1>3}$ antagonist VPC44116 (Sim-Selley et al 2009). Moreover, the $S1P_1$-selective agonist SEW2871 (i.c.v.) also produced thermal antinociception that was blocked by VPC44116. S1P was also administered with the CB_1 antagonist rimonabant because cannabinoids produce a similar tetrad of effects (Compton et al 1991) and sphingosine derivatives interact with CB_1Rs (Paugh et al 2006). However, rimonabant pretreatment did not inhibit these S1P-mediated effects, suggesting that S1P is a parallel system to the eCBs. Intraperitoneal (i.p.) administration of FTY720, which efficiently crosses the blood-brain barrier (Meno-Tetang et al 2006), produces similar *in vivo* effects (Welch et al 2009), suggesting that peripheral administration of S1PR pro-drugs produces antinociception via CNS mechanisms. These findings support a role for central S1PRs in antinociception, and also indicate that motor side effects might limit the therapeutic utility of non-selective S1P agonists. Importantly, $S1P_1$Rs were implicated specifically in antinociception, suggesting that development of type-specific agonists might minimize unwanted side effects of these drugs.

S1P effects on acute nociception

As discussed above, central administration of S1P produced thermal antinociception in the tail-flick test in mice, apparently via an $S1P_1$R-mediated mechanism (Sim-Selley et al 2009). Similarly, (Coste et al 2008a) reported that intrathecal (i.t.) administration of sphingosine kinase inhibitors reduced nociceptive latencies in the hotplate test in rats,

presumably by reducing endogenous S1P synthesis. Moreover, SphK 2 null mice exhibited lower hotplate latencies compared to wild-type mice. These results suggest that endogenous S1P in the spinal cord tonically inhibits thermal nociceptive sensitivity. Further support for this hypothesis has been obtained in inflammatory pain models (see below). Together, our findings and those of Coste et al. indicate that S1P produces thermal antinociception via $S1P_1$Rs in the CNS.

S1P agonists in chronic or inflammatory pain

S1P could regulate peripheral and central pain pathways at multiple points, therefore its role in nociception and potential for therapeutic development can only be fully evaluated using multiple experimental paradigms in the intact organism. Indeed, studies to date suggest that S1P can either inhibit or enhance nociception in models of inflammatory or chronic pain depending upon pain models, species, and dose, route and timing of drug administration. Several studies have investigated S1P ligands in models of inflammatory pain. Coste et al (2008a) reported that formalin- or zymosan-induced inflammation of the rat paw decreased S1P levels in the cerebrospinal fluid, which was associated with enhanced pain sensitivity. Conversely, S1P or dihydro-S1P (i.t.) administration reduced pain-related behavior in the formalin test. These results suggested S1PR mediated antinociception because dihydro-S1P acts as an S1PR agonist but does not produce other intracellular effects of S1P. In agreement with this interpretation, antinociception was associated with S1P-induced inhibition of cAMP synthesis in excitatory neurons of spinal cord. Moreover, formalin enhanced phosphorylation of the NMDA NR1 subunit and calmodulin kinase II in these spinal neurons, and this phosphorylation was reversed by i.t. administration of dihydro-S1P. These results suggest that S1PR-mediated inhibition of cAMP and protein phosphorylation reduced nociceptive processing in the spinal cord. Overall, these findings are reminiscent of results from the eCB system, where genetic knockdown or pharmacological blockade of CB_1Rs was associated with NMDA-dependent hyperalgesia (Richardson et al 1998).

In a separate study, (Coste et al 2008b) reported that both i.p. and i.t. administration of FTY720 produced antinociception in the rat formalin assay. Similarly, mechanical and thermal (cold) allodynia in the rat sciatic nerve ligation model of neuropathic pain was inhibited by acute peripheral administration of FTY720 (Coste et al 2008b). Although FTY720 can inhibit phospholipase A_2 (Payne et al 2007), it did not alter spinal prostaglandin synthesis in this study, and was likely acting via S1PRs. Surprisingly, SEW2871 (i.p. or i.t.) did not mimic FTY720 in this study, but because only low SEW2871 doses were administered, it is possible that $S1P_1$R occupancy was insufficient to produce antinociception. In contrast, we showed that i.c.v. administration of SEW2871 produced thermal antinociception that was blocked by VPC44116 (Sim-Selley et al 2009). Nonetheless, S1PR antagonists were not examined by Coste et al. (2008b), so it is conceivable that FTY720-P (but not SEW2871) inhibited inflammatory or neuropathic pain by functional antagonism of one or more S1PR types, as discussed above.

Additional mechanisms besides S1PR activation are likely to be involved in sphingolipid regulation of nociception. For example, metabolomics analysis in a rat nerve transection model of neuropathic pain revealed significantly increased levels of the sphingosine

metabolite DMS, along with ceramide, sphingosine and sphinganine, in the spinal cord (Patti et al 2012). Moreover, i.t. injection of DMS in rats increased several inflammatory markers associated with glial activation, supporting a role for DMS in nociceptive hypersensitivity. In contrast, neither nerve-transection nor DMS altered S1P levels, which is somewhat surprising because DMS is a SphK inhibitor. As the authors noted, however, the DMS levels detected were significantly lower than required to inhibit SphK activity, suggesting that an alternative target could mediate the effect of DMS on nociceptive hypersensitivity. We recently observed that DMS and sphinganine act as CB_1R antagonists (Selley et al 2011). Furthermore, the eCBs can produce CB_1-mediated analgesia in neuropathic pain models (Guindon & Hohmann 2009, Kinsey et al 2010, Walker et al 2002). Therefore, if local concentrations of sphingosine, sphinganine and DMS were sufficient to block CB_1Rs, they might inhibit the eCB system in this model. However, this presupposes that eCB-CB_1 receptor interactions play a tonic role in modulating neuropathic pain, which might not be the case (Kinsey et al 2010).

In contrast to the findings discussed above, S1PR activation has also been reported to produce peripheral hyperalgesia/sensitization following intradermal injection of S1P directly into the rat paw (Doyle et al 2011b). This effect was probably $S1P_1R$-mediated because it was mimicked by SEW2871 and blocked by local administration of the $S1P_{1>3}$ antagonist W146, but not its inactive isomer. Similarly, hyperalgesic effects of locally administered ceramide were also blocked by W146, SphK inhibitors or an anti-S1P antibody (Doyle et al 2011a), suggesting that ceramide-induced hyperalgesia resulted from locally produced S1P acting via $S1P_1Rs$. A subsequent study by this group demonstrated a role for $S1P_1R$-mediated neutrophil activation in hyperalgesia elicited by intraplantar injection of carrageenan or S1P (Finley et al 2013). However, a recent study examined the effect of intraplantar zymosan injection on S1P levels in mice, and found decreased S1P in the inflamed paw and no change in plasma or whole blood S1P levels (Linke et al 2012).

Further support for peripheral nociceptive sensitization by S1P was obtained by local perfusion of S1P into the rat dorsal root ganglia (DRG), which produced ~50% increase in sensitivity to mechanical stimulation of the paw (Xie et al 2012). Similarly, perfusion of explanted DRG with S1P increased sensory neuron firing rates. Moreover, zymosan perfusion into DRG greatly increased mechanical sensitivity, which was partially inhibited by administration of siRNA against the $S1P_1R$, suggesting its involvement in the hypersensitivity response. This work supported earlier findings that $S1P_1Rs$ directly enhanced sensory neuron sensitivity (Chi & Nicol 2010). In that study, S1P and SEW2871 enhanced the electrical excitability of 50% of cultured small diameter sensory neurons expressing $S1P_{1–4}R$ mRNA. However, not all excitatory effects of S1P on these neurons were $S1P_1R$-mediated because approximately 25% of S1P-responsive neurons were not responsive to SEW2871 (Chi & Nicol 2010). Moreover, intracellular application of S1P, which can modulate non-GPCR targets, also produced hyperexcitability in cultured sensory neurons (Zhang et al 2006). Thus, S1P might promote peripheral nociceptive sensitization via mechanisms involving both $S1P_1Rs$ and other S1P targets.

A similar conclusion was reached in another study that showed an excitatory role of S1P acting via $S1P_1Rs$ and a non-$S1P_1R$-mediated mechanism in peripheral pain sensitization

(Mair et al 2011). Mice with and without conditional genetic deletion of $S1P_1$Rs in primary nociceptive neurons were injected with S1P, SEW2871 and/or the TRPV1 agonist capsaicin into the paw, and thermal hyperalgesia and nociceptor neuron activity were assessed. In addition, mRNA analysis in DRG explants showed $S1P_{1–3}$R expression in sensory neurons. SEW2871 augmented the nociceptive actions of capsaicin in control but not $S1P_1$R conditional null mice, indicating that $S1P_1$Rs enhanced TRPV1-mediated nociception. Interestingly, $S1P_1$R deletion reduced the hypersensitivity induced by 100 μM but not 10 μM S1P. The authors concluded that S1P might be acting at multiple sites in both immune and neural cells to enhance nociceptive sensitivity. More recently, this group reported that $S1P_3$Rs predominate on DRG neurons, and contribute to hyperexcitation by activating an excitatory chloride conductance (Camprubi-Robles et al 2013). Moreover, both S1P-induced nociceptor excitation and spontaneous pain behaviors were reduced in $S1P_3$R global null mice, but not in mice with conditional $S1P_1$R deletion in primary nociceptors, thus identifying $S1P_3$Rs as major S1P targets in peripheral sensitization.

As noted by Mair et al. (2011), the dichotomy of FTY720- or S1P-induced analgesia centrally versus S1P-induced hyperalgesia peripherally is reminiscent of eCBs. Cannabinoids can produce analgesic effects via CB_1Rs expressed on peripheral nociceptors (Agarwal et al 2007) or hyperalgesic effects in the spinal cord where they mediate C-fiber-induced heterosynaptic pain sensitization via CB_1Rs expressed on GABAergic interneurons (Pernia-Andrade et al 2009). Thus, both CB_1Rs and S1PRs can exhibit opposing effects on pain in the peripheral nervous system (PNS) versus CNS. Nonetheless, direct i.t. administration of cannabinoids produces antinociception and antihyperalgesia (Welch & Stevens 1992). Thus, the predominant effect of systemically or centrally administered S1PR or CB_1R agonists appears to be antinociceptive, despite demonstrated pro-nociceptive roles of both endogenous systems in certain cell populations and pain models.

Conclusions

The importance of lipid mediators in pain modulation is widely recognized. In addition to the established roles of prostaglandins in inflammatory pain, the eCB system modulates pain states that include acute, inflammatory and chronic neuropathic pain. Evidence supporting a critical regulatory role of the eCB system in various pain states is growing rapidly, and this system is a promising target for development of analgesics. Likewise, emerging evidence suggests that sphingolipid-derived mediators, such as S1P, are also critical regulators of pain. S1P and $S1P_1$R agonists produce antinociception in acute thermal pain via CNS-mediated actions. The $S1P_{1,3,4,5}$ agonist pro-drug, FTY720 also inhibits inflammatory and neuropathic pain, but the mechanism of these effects is not yet known. S1P in the PNS sensitizes primary nociceptive neurons through a mechanism that involves $S1P_1$Rs, $S1P_3$Rs and possibly other targets. Moreover, S1PRs are expressed in immune cells and glia, which can also modulate nociceptive sensitivity and are likely to play crucial roles in inflammatory and chronic pain states.

Additional sphingolipid-derived compounds that are S1P precursors or metabolites can also contribute to pain regulation. For example, DMS, sphingosine and sphinganine levels were elevated in the spinal cord in a chronic neuropathic pain model. These compounds are CB_1R

antagonists that might oppose the eCB system, although whether they reach sufficient levels locally to physiologically inhibit eCB function remains to be determined. Moreover, it is possible that these compounds act at additional, as yet unidentified targets.

Altogether, current evidence supports the conclusion that the endogenous sphingolipid system regulates pain, and might interact with the eCB system in the PNS, CNS and immune system. Thus, future challenges will be to determine both the local physiological and pathophysiological levels of sphingolipids and their metabolites in chronic pain states and their effects on relevant target sites of action. A more detailed understanding of these systems could ultimately lead to the development of novel pharmacotherapies to treat pain.

Acknowledgments

The authors thank the following for supporting this work: Virginia Commonwealth University Presidential Research Initiative Proposal (PRIP) grant (Welch); Thomas F. and Kate Miller Jeffress Memorial Trust grant (Sim-Selley) and NIDA grants R01 DA014277 and DA030404 (Sim-Selley).

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