REVIEW

Anandamide-Mediated Modulation of Nociceptive Transmission at the Spinal Cord Level

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Summary

Three decades ago, the first endocannabinoid, anandamide (AEA), was identified, and its analgesic effect was recognized in humans and preclinical models. However, clinical trial failures pointed out the complexity of the AEA-induced analgesia. The first synapses in the superficial laminae of the spinal cord dorsal horn represent an important modulatory site in nociceptive transmission and subsequent pain perception. The glutamatergic synaptic transmission at these synapses is strongly modulated by two primary AEA-activated receptors, cannabinoid receptor 1 (CB_1) and transient receptor potential vanilloid 1 (TRPV1), both highly expressed on the presynaptic side formed by the endings of primary nociceptive neurons. Activation of these receptors can have predominantly inhibitory (CB_1) and excitatory (TRPV1) effects that are further modulated under pathological conditions. In addition, dual AEA-mediated signaling and action may occur in primary sensory neurons and dorsal horn synapses. AEA application causes balanced inhibition and excitation of primary afferent synaptic input on superficial dorsal horn neurons in normal conditions, whereas peripheral inflammation promotes AEA-mediated inhibition. This review focuses mainly on the modulation of synaptic transmission at the spinal cord level and signaling in primary nociceptive neurons by AEA *via* CB_1 and TRPV1 receptors. Furthermore, the spinal analgesic effect in preclinical studies and clinical aspects of AEA-mediated analgesia are considered.

Key words

Anandamide • CB_1 • TRPV1 • NAPE • Spinal cord • Synaptic transmission

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Introduction

Pain modulation and analgesic effects of the endocannabinoid anandamide (AEA, *N*-arachidonoyl-ethanolamine) were recognized in humans and intensively studied in experimental rodent models [1-7]. However, the underlying mechanisms of AEA anti-nociceptive action still need to be understood better due to the complexity of the AEA metabolism, trafficking and storage, the whole endocannabinoid system balance, and AEA dual effects on sensory processing and signaling [8-10]. The scientific effort to understand and modulate AEA-mediated signaling at the spinal cord level has substantial implications for the development of new possible therapeutic strategies for pain relief.

Anandamide as a part of the endocannabinoid system and endocannabinoidome

The endocannabinoid system consists of classical cannabinoid CB_1 and CB_2 receptors, endo-cannabinoids – AEA and 2-arachidonoylglycerol (2-AG), and their synthesizing and degradation enzymes [11]. It exerts homeostatic function and controls a wide range of various physiological roles like emotional processing, learning and memory, sleep, appetite, cardiovascular functions, reproduction, temperature control, immune

response, inflammation and pain. The essential role of the endocannabinoid system in CNS is neuromodulation, affecting synaptic plasticity, as well as nociceptive synaptic transmission [12]. Imbalance or malfunction of endocannabinoid system is involved in nervous system disorders such as anxiety, depression, schizophrenia, multiple sclerosis, neurodegeneration, stroke, epilepsy, addiction, and pathological pain states [13-15].

Lately, a new concept of endocannabinoidome has been established (Fig. 1). The endocannabinoidome extends the boundaries of the endocannabinoid system to include endocannabinoid-like lipid mediators that are biochemically related to the endocannabinoids. These lipids include a family of *N*-acylethanolamines (NAEs), including *N*-palmitoylethanolamine (PEA), *N*-oleoylethanolamine (OEA), *N*-linoleoylethanolamine (LEA), *N*-docosahexaenoylethanolamine (DHEA), a family of 2-acylglycerols (2-oleoyl glycerol, 2-OG; 2-linoleoyl glycerol, 2-LG), *N*-acyl neurotransmitters (*N*-acyl dopamines, *N*-acyl serotonins) or also lipoamino acids (*N*-acyl taurines, *N*-acyl glycines). The endocannabinoidome further encompasses enzymes for the mentioned bioactive lipids syntheses and degradation, and their receptors, among others, orphan G-protein coupled receptors (GPR55, GPR110, GPR18 or GPR119), one of the key nociceptive receptors – transient receptor potential vanilloid 1 (TRPV1) or nuclear peroxisome proliferator-activated receptors PPARα and PPARγ [1]. Changes in AEA metabolism/level within the wider endocannabinoidome may alter metabolic pathways of other lipid mediators and modulate alternative signaling with an impact on physiological functions. The endocannabinoidome exerts a more comprehensive impact on health and body homeostasis and thus should also be considered in clinical studies related to AEA.

AEA is an arachidonic acid derivative, *N*-arachidonoylethanolamine, a naturally occurring compound within the body belonging to the larger family of NAEs. It was the first endocannabinoid identified from the porcine brain and was later isolated and measured in humans and rats [16,17]. The word Ananda means bliss and happiness in Sanskrit, which fits well with current research that describes AEA-mediated signaling through the CB_1 receptor to produce analgesic, anxiolytic, and antidepressant effects [7,18-20].

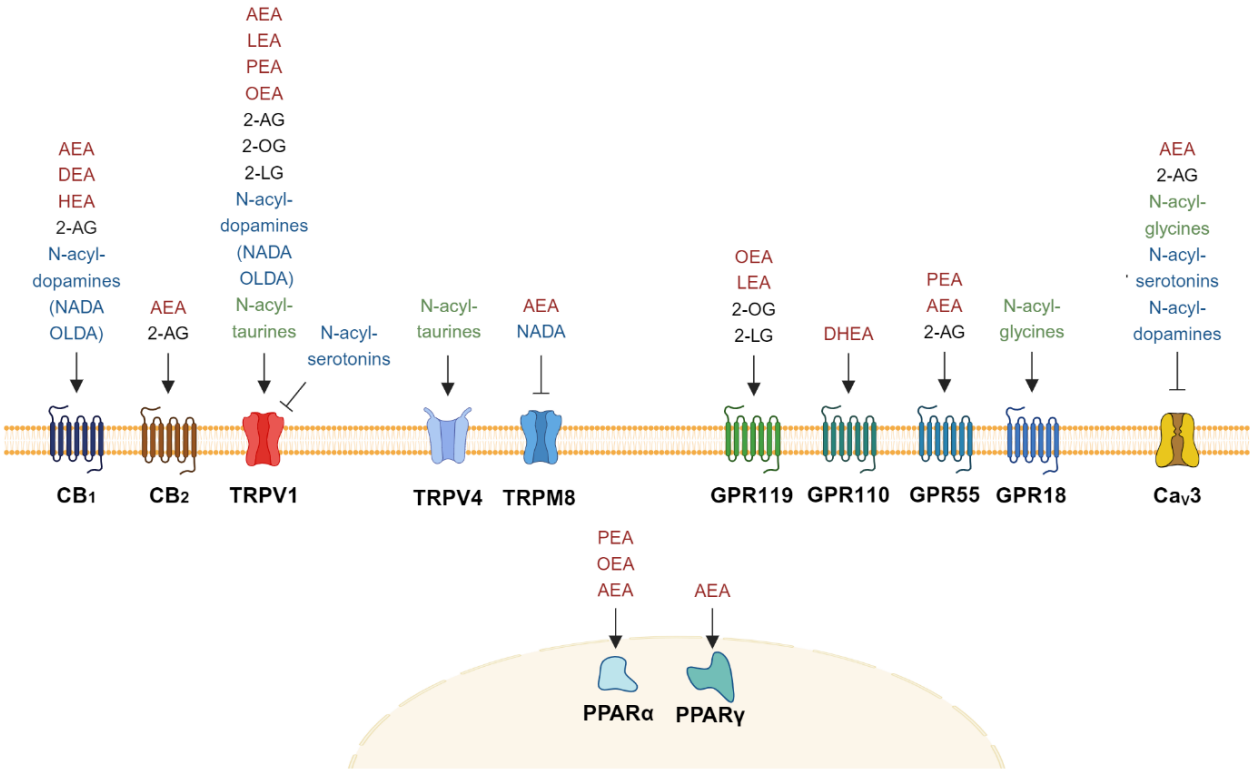

Fig. 1. Endocannabinoidome receptors and mediators. Groups of endocannabinoid-like lipid mediators are distinguished in colors: *N*-acylethanolamines in red, 2-acylglycerols in black, *N*-acyl neurotransmitters in blue, and lipoamino acids in green. Abbreviations: 2-AG, 2-arachidonoylglycerol; AEA, *N*-arachidonoylethanolamine (anandamide); DEA, *N*-docosatetraenoylethanolamine; DHEA, *N*-docosahexaenoylethanolamine; HEA, *N*-homo-γ-linolenylethanolamine; LEA, *N*-linoleoylethanolamine; NADA, *N*-arachidonoyldopamine; OEA, *N*-oleoylethanolamine; OLDA, *N*-oleoyldopamine; PEA, *N*-palmitoylethanolamine. The image was created with BioRender.com.

AEA was initially identified to bind preferentially to the CB_1 receptor and with lower affinity to the CB_2 receptor [16]. Later, the activation of nonselective cation channel TRPV1 by AEA was recognized, while its efficacy at human TRPV1 was reported to be higher than that at rat TRPV1 [21,22]. Thus, AEA acts as an endocannabinoid and endovanilloid; the CB_1 and TRPV1 being the main AEA activated receptors. Other molecular targets affected by AEA were also recognized – including nuclear receptors PPARγ and PPARα, voltage-gated T-type calcium channels (Ca_V3) and sodium channels [23-27]. AEA activation of both CB_1 and TRPV1 receptors at the first nociceptive synapse in the spinal cord dorsal horn plays an important role in pain modulation. The action of AEA at this synapse is complex, as the effect of presynaptic CB_1 receptor activation on neurotransmitter release is inhibitory, and the main impact of TRPV1 activation is excitatory.

Anandamide metabolism

Under neuronal stimulation, AEA is produced in and released from neurons in a Ca^{2+}-dependent manner [28]. Furthermore, the Ca^{2+}-independent formation of AEA was later demonstrated after PKC and PAK activation in primary sensory neurons, also named dorsal root ganglion (DRG) neurons [29]. Redundant biosynthetic pathways of AEA were characterized; phospholipase A2 group IV E (PLA2G4E, cPLA2ε) was identified as Ca^{2+}-dependent N-acyltransferase (Ca-NAT), which catalyzes the formation of AEA precursor *N*-acylphosphatidylethanolamine (NAPE) from phosphatidylethanolamine (PE) and phosphatidylcholine (PC), with a transfer of the acyl group of PC to the amine of PE [30]. In brain lysate, Ca-NAT activity preferably generates N-arachidonoyl-containing (p)NAPEs with polyunsaturated acyl groups at the sn-2 position [1,31]. On the other hand, Ca^{2+}-independent N-acyltransferases (NATs) termed phospholipase A and acyltransferase (PLAAT) can also generate NAPE [32,33]. The main enzyme converting NAPE to AEA is *N*-acylphosphatidylethanolamine phospholipase D (NAPE-PLD), which catalyzes the AEA syntheses in a Ca^{2+}-sensitive manner [34]. But, AEA may also be synthesized from NAPE by other Ca^{2+}-insensitive enzymes [35].

In the CNS, the enzyme fatty acid amino hydrolase (FAAH) primarily degrades AEA to arachidonic acid and ethanolamine. It is important to note that it accepts multiple fatty acid amides as a substrate, including palmitoyl- and oleoyl-ethanolamide [36]. In addition to the hydrolytic pathway, AEA undergoes oxygenation by cyclooxygenase 2 (COX-2), 5-, 12-, 15-lipoxygenase (LOX) and cytochrome P450 monooxygenases to create prostaglandin-ethanolamides, hydroxyeicosatetraenoyl-ethanolamides (HETE-EAs) and epoxyeicosatrienoyl-ethanolamide see for reviews [1,8].

Interaction between CB_1 receptor and TRPV1 channel signaling in DRG neurons

In nociceptive DRG neurons, an opposite role for CB_1 and TRPV1 receptor activation was suggested, while AEA may activate both and trigger complex effects. AEA acts as a low-efficacy TRPV1 agonist. The efficacy of AEA may be affected by the number of TRPV1 channels in the plasmatic membrane, their phosphorylation, AEA metabolism, efflux or uptake, trafficking, storage, and the critical role also plays the concomitant CB_1 receptor activation [8,37-41]. The AEA-mediated effects may differ based on the expression of the target receptors. Neurons expressing only CB_1 receptors or only TRPV1 channels may exert the opposite AEA-induced effects. In neonatal rat cultured neurons, the AEA-mediated inhibitory or excitatory effect was reported in size-segregated DRG populations of neurons. In supporting experiments, Ca^{2+} transients were evoked by KCl-induced depolarization and AEA application inhibited or potentiated these Ca^{2+} transients [42]. The CB_1 activation-mediated inhibitory effect on depolarization-induced Ca^{2+} transients was also shown in primary culture of adult DRG neurons, which additionally rarely responded to capsaicin [43]. The dual effect of AEA application on synaptic input from primary nociceptive fibers was demonstrated at the first nociceptive synapses formed by these nociceptive DRG endings in acute spinal cord slices [10].

However, CB_1 and TRPV1 receptors exhibit high co-expression within the DRG neurons [44-46], suggesting complex AEA-mediated regulation based on CB_1/TRPV1 crosstalk in these neurons. In transfected cells over-expressing both receptors, a dual effect of CB_1 activation on capsaicin evoked, TRPV1-mediated Ca^{2+} response was described. Activation of the CB_1 receptor inhibited or enhanced capsaicin-induced responses depending on the signaling pathways activated. Inhibition of TRPV1 channel responses was mediated by negative regulation of the cAMP signaling *via* CB_1 receptor activation. The opposite effect, potentiation of TRPV1 channel responses, was mediated *via* phosphatidylinositol 3-kinase (PI3K) and

phospholipase C (PLC) pathways stimulated by CB_1 receptor activation [47]. Meanwhile, the activation of diverse intracellular signaling cascades, such as PI3K, PLC-PKC or PKA are known to sensitize the TRPV1 channel to the agonist [38,41,48-53]. Further, it was shown that the constitutive activity of the CB_1 receptor maintains the TRPV1 channel in a sensitized state [54]. The effect of AEA thus depends on the activation/regulation of CB_1 and TRPV1 receptors and the concomitant CB_1-mediated regulation of specific intracellular signaling with an impact on the TRPV1 sensitization. The order in which individual receptors are activated could also play a role [47,54]. A later study suggested that CB_1-supported TRPV1 sensitization and the induction of TRPV1 responsiveness to AEA in DRG neurons may be underlined by the spatial proximity of both receptors [55].

On the other hand, reduction of TRPV1 sensitization after CB_1 receptor activation was also shown in experiments where AEA in subthreshold concentration for TRPV1 activation facilitated heat-induced Ca^{2+} transients that were further enhanced by CB_1 receptor inhibition. The Ca^{2+} transients induced by AEA in the concentration to activate TRPV1 were reduced by simultaneous CB_1 receptor activation by CB_1 agonist HU210 [56]. Capsaicin evoked TRPV1-mediated cationic influx was attenuated by CB_1 receptor activation, which in addition also reduced the number of capsaicin-responsive cells in these experiments [40,57]. Remarkably, experiments in spinal cord slices showed that AEA induced a concentration-dependent release of neuropeptide, calcitonin gene-related peptide (CGRP) and substance P (SP), *via* TRPV1 channel activation on central terminals of DRG neurons [58]. This TRPV1-mediated pronociceptive process of neuropeptide release in the dorsal horn was reduced by CB_1 activation [59]. Thus, AEA activation of CB_1 receptors on central terminals of DRG neurons may concomitantly affect the sensitization/activation of TRPV1 channels, and the final AEA effect could be concentration and receptors proximity dependent. In addition, the increase in intracellular Ca^{2+} concentration in DRG neurons stimulates the formation of endogenous AEA. Newly synthesized AEA was shown to mediate TRPV1-dependent Ca^{2+} influx subsequently. Thus, AEA was proposed to act as an intracellular messenger, amplifying intracellular concentration of Ca^{2+} *via* TRPV1 channels [39].

A fatty acid binding protein 5 (FABP5) is an intracellular carrier for AEA transport to FAAH-mediated hydrolyses. A conditional knockout strategy was used to selectively ablate FABP5 in the TRPV1 channel expressing DRG neurons. This genetic approach elevated AEA, PEA, and OEA levels in DRGs, while 2-AG levels remained unchanged. Elevated AEA levels in nociceptive DRGs after FABP5 deletion attenuated nerve growth factor-mediated TRPV1 sensitization *via* CB_1 receptor activation, and the emergence of antinociceptive effects mediated by CB_1 was thus revealed [60].

CB_1 and TRPV1 receptors modulation of transmitter release in spinal cord dorsal horn

The first synapses of the pain pathway are localized in the spinal cord dorsal horn, particularly in the superficial laminae. Nociceptive signaling from the periphery is transmitted from DRG neurons, to dorsal horn neurons, which convey signaling to higher brain areas. AEA acts on the CB_1 and TRPV1 receptors expressed at presynaptic endings of primary afferents and modulate neurotransmitter release. Activation of presynaptic CB_1 receptors decreases glutamate release by a well-established mechanism of trimeric $G_{i/o}$-protein cascade stimulation, inhibiting adenylyl cyclase, decreasing calcium conductance by inhibition of high-voltage activated N- and P/Q-type Ca^{2+} channels, and increasing the potassium conductance *via* stimulation of inwardly rectifying and A-type outward potassium channels (Fig. 2) [61,62]. In comparison, CB_1 receptor coupling to G_s-protein, stimulating adenylyl cyclase, was unmasked when the $G_{i/o}$-protein cascade was inhibited [63]. Whereas the isoform of adenylyl cyclase expressed in cells may be crucial in the CB_1 receptor activation-induced dual effect on adenylyl cyclase [64].

TRPV1 activation-mediated responses are characterized by two phenomena – desensitization and tachyphylaxis. During TRPV1 stimulation, the channel activity is Ca^{2+}-dependently reduced, and TRPV1 thus undergoes rapid desensitization. Tachyphylaxis occurs during repetitive agonist stimulation while the TRPV1-mediated responses are diminished. TRPV1 channel activation at presynaptic ending allows Ca^{2+} influx through the opened pore, increasing Ca^{2+} concentration in the cytosol and dramatically enhancing spontaneous glutamate release [65-67]. Potent TRPV1 agonist capsaicin application elicited action potentials in superficial dorsal horn neurons, but evoked glutamate release induced by electrical stimulation of the dorsal root was prevented [65,66]. Thus, the facilitation of spontaneous glutamate release by capsaicin was sufficient to transmit nociceptive information further along the pain pathway by activating

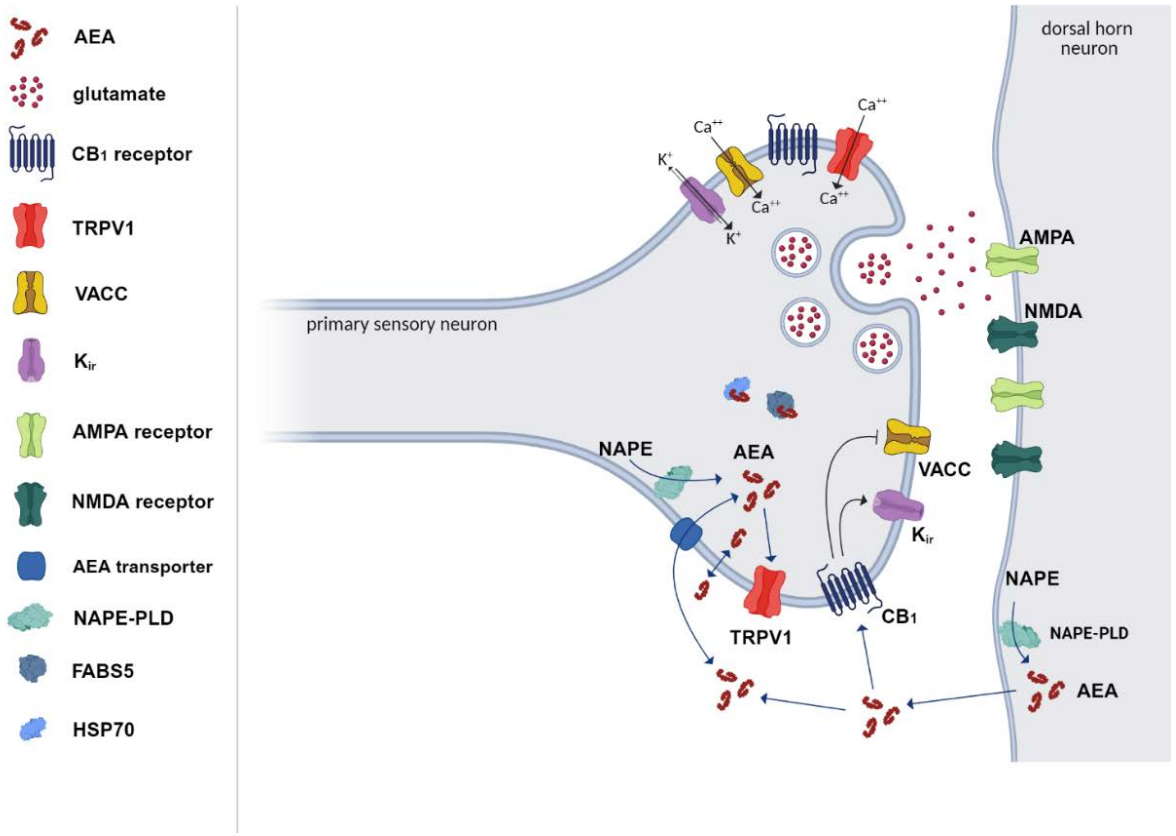

Fig. 2. Simplified illustration of AEA action at the first nociceptive synapse formed by the central terminal of the primary sensory neuron and the secondary spinal cord dorsal horn neuron. Autocrine and retrograde signaling of AEA is suggested, whereas AEA is synthesized in addition to other enzymes by Ca^{2+}-dependent NAPE-PLD in both primary and secondary nociceptive neurons. (The contribution of glial cells to AEA signaling is not depicted). Translocation of the lipid molecule of AEA across the plasma membrane by diffusion and a membrane transporter was proposed. Intracellular carriers, including the fatty acid binding protein 5 (FABP5) and the heat shock protein 70 (HSP70), may facilitate the intracellular transport of AEA, for example, towards the FAAH for degradation. Two primary AEA-activated receptors are abundantly expressed on the presynaptic side, where they regulate glutamate release. At lower concentrations, the CB_1 receptor is suggested to be activated, while at higher concentrations, both the CB_1 and TRPV1 receptors are activated. In addition, AEA could directly inhibit low-voltage-activated calcium channels (Ca_V3) to modulate the excitability of neuron. The reduction in transmitter release after CB_1 receptor activation is attributed to the inhibition of high-voltage-activated calcium channels (VACC) and the activation of inwardly rectifying potassium channels (K_{ir}). The image was created with BioRender.com.

second-order neurons even when the action potential-evoked glutamate release from primary afferent endings was blocked. Furthermore, *in vivo* electrophysiological experiments demonstrated that spinal administration of TRPV1 antagonist capsazepine reduced nociceptive fibers (Aδ- and C-) stimulation-evoked responses of dorsal horn neurons [68]. It is suggested that endogenous AEA primarily activates CB_1 receptors under normal conditions, and its concentration is insufficient for TRPV1 channel stimulation on the central endings of DRG neurons.

Modulation of synaptic transmission at first nociceptive synapses by anandamide

In the superficial spinal cord dorsal horn, the patch-clamp recording of the dorsal root electrical stimulation-evoked excitatory postsynaptic currents (eEPSCs) from lamina II neurons revealed inhibition of the eEPSC amplitude by AEA application. Meanwhile, AEA attenuated evoked excitatory transmission more effectively during Aδ-fiber than C-fiber stimulation. A similar decrease of eEPSC amplitude was demonstrated after the CB_1 receptor agonist WIN55,212-2 application [69,70]. The effects of AEA on spontaneous synaptic transmission were reported inconsistently, from no detected change in the frequency of miniature EPSC (mEPSC) [70] to a concentration-dependent effect of AEA [71]. In the latter experiments, mEPSCs were recorded, and the low AEA concentration-induced inhibition of frequency was suggested to be mediated *via* CB_1 receptors activation, and the higher AEA concentration-induced excitatory effect *via* TRPV1 channels [71].

A recent study evaluated AEA modulation by

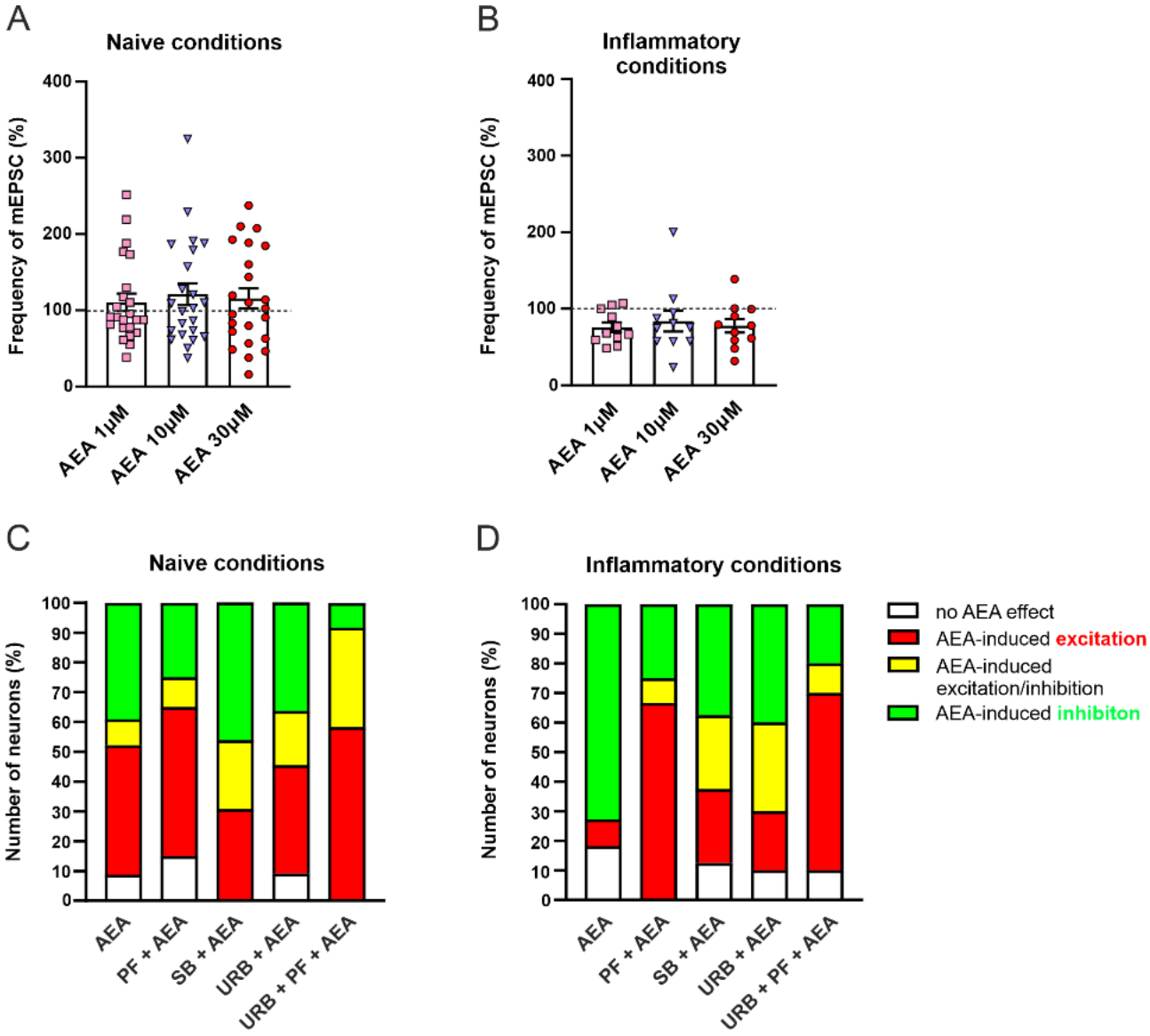

Fig. 3. Peripheral inflammation enhanced the inhibitory effect of AEA application on dorsal horn neurons mEPSC frequency in spinal cord slices. (**A, B**) Normalized frequency of mEPSC during acute AEA application (1 μM, 10 μM, and 30 μM, 4 min each concentration) in control conditions (A) and 24 h after induction of peripheral inflammation (B) by subcutaneous carrageenan injection. Statistical analysis showed a significant difference between control and inflammatory conditions in each AEA concentration tested (1 μM AEA, $p<0.05$; 10 μM AEA, $p<0.05$; 30 μM AEA, $p<0.05$). (**C**) Application of AEA induced decrease or increase of mEPSC frequency in a comparable number of superficial dorsal horn neurons in control conditions. The number of neurons with mEPSC frequency inhibition by AEA application was reduced after inhibition of the CB_1 receptor (PF514273 application) and especially after CB_1 and FAAH co-inhibition (PF514273/URB597 co-application) used as a pretreatment. (**D**) After peripheral inflammation, most neurons received synaptic input inhibited by the AEA application (73 %). Pretreatment with PF514273 and PF514273/URB597 reduced the inhibitory and enhanced the excitatory AEA-induced effect. Abbreviations: PF (PF514273, CB_1 receptor antagonist), SB (SB366791, TRPV1 antagonist), URB (URB597, FAAH inhibitor). The figure was adapted from Pontearso *et al.* [10].

recording mEPSC from neurons in lamina I and $II_{(outer)}$ in acute slices [10]. The results suggested that applied AEA had a dual effect on mEPSC frequency with similar size populations of recorded neurons showing inhibition and excitation. This balanced AEA effect on mEPSC frequency was changed following peripheral inflammation when AEA-induced decrease of neurotransmitter release from primary afferent fibers (mEPSC frequency) was dominant (Fig. 3). The excitatory effect of AEA application was evident only when CB_1 receptors and FAAH were inhibited [10]. Notably, these described effects of exogenous AEA application contrast with those observed following the application of its precursor 20:4-NAPE, which increased levels of endogenous AEA and consistently inhibited both action potential-dependent and -independent excitatory synaptic transmission, as evidenced by the recording of

eEPSC, sEPSC, and mEPSC [9,72]. In these experiments, AEA was synthesized in spinal cord slices from 20:4-NAPE primarily by NAPE-PLD (Fig. 4) [72]. The 20:4-NAPE mediated inhibition was also present after peripheral inflammation while the underlying mechanisms were altered. In naïve animals, the 20:4-NAPE effect was mediated by CB_1 receptors, but after inflammation, the TRPV1 channel-mediated mechanism was also involved [9,72]. The physiological mechanism of AEA syntheses by available catabolic enzymes, together with their cellular distribution and level of enzymatic activity, regulate AEA concentration locally and may have a crucial role in the AEA-induced modulation of nociception [9]. The local production of AEA from its precursor could thus be advantageous for analgesic purposes in clinical settings.

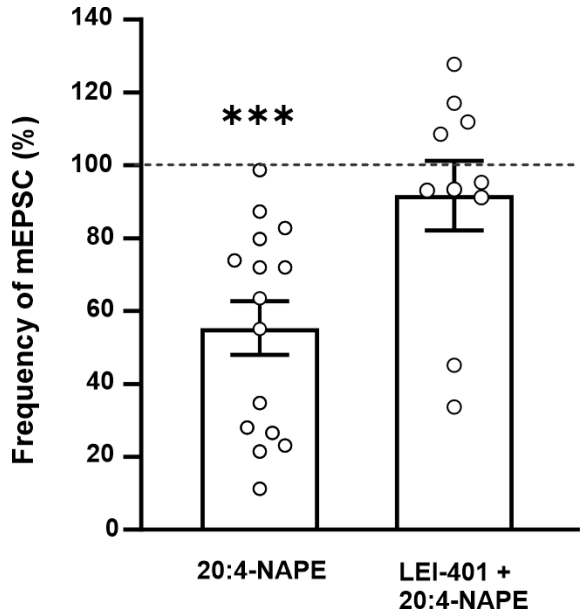

Fig. 4. Application of the AEA precursor 20:4-NAPE decreased excitatory synaptic transmission at the first nociceptive synapses *via* NAPE-PLD activation. Application of 20:4-NAPE (20 μM, 4 min) decreased the frequency of mEPSC (n = 15, *** p<0.001) recorded from superficial dorsal horn neurons in acute spinal cord slices. Incubation of slices with the NAPE-PLD inhibitor LEI-401 (1 μM, 2 h) prevented the effect of acutely applied 20:4-NAPE (20 μM, 4 min) on mEPSC frequency. The figure was adapted from Spicarova *et al.* [72].

Well-known endocannabinoid retrograde signaling described at synapses in the brain was also recognized in the spinal cord dorsal horn. Activation of spinal metabotropic glutamate receptor 5 ($mGluR_5$) stimulated endocannabinoid-mediated stress-induced analgesia by retrograde signaling *via* diacylglycerol lipase – 2-arachidonoylglycerol – CB_1 receptor pathway [73]. In other experiments different conditioning stimulation protocols known to induce endocannabinoid production and CB_1 receptor-dependent synaptic plasticity in other brain areas [74,75] were employed in spinal cord slices. Low-frequency stimulation of primary afferent fibers combined with depolarization of postsynaptic neuron led to profound long-term depression mediated by CB_1 receptors [76]. These results indicated that CB_1 receptors activation in primary afferent fibers could prevent long-term potentiation underlying hypersensitive states.

Various populations of excitatory and inhibitory spinal interneurons form neuronal circuits in the dorsal horn and modulate the nociceptive signaling from the periphery. This signaling is also affected by descending modulation from higher brain areas. Many studies aimed at spinal nociception are performed in spinal cord slices. This preparation decreases the degree of complexity of spinal nociceptive signaling by eliminating functional descending pathways. Despite the expression on central terminals of primary sensory neurons, CB_1 receptors are also expressed in dorsal horn interneurons [44,73,77-79]. Their activation may decrease the inhibitory neurotransmitter release, leading to increased excitability of nociceptive dorsal horn neurons. Thus, an unexpected role of endocannabinoids acting on inhibitory interneurons as mediators of heterosynaptic pain sensitization was revealed in the dorsal horn [77].

Analgesia mediated by spinal anandamide

Treatment with cannabinoids induces analgesia by acting at the peripheral, spinal, and supraspinal levels [2,4,18,80,81]. Intrathecal (i.t.) administration of the CB_1 receptor antagonist, SR141716A, induced thermal hyperalgesia and facilitated responses of dorsal horn neurons evoked by transcutaneous electrical stimulation. These experiments suggested tonic activation of spinal CB_1 receptors modulating nociceptive threshold [82,83]. In comparison, i.t. administration as well as topical application of CB_1 receptor agonist WIN55,212-2 produced analgesia. When ineffective i.t. doses of WIN55,212-2 were used with topical tail immersion in the WIN55,212-2 solution, an antinociceptive effect was markedly potentiated. Thus, antinociceptive synergy occurred in both peripheral and spinal application sites [84]. Spinal administration of AEA had inconsistent effects on neuronal responses evoked by transcutaneous electrical stimulation of nociceptive primary afferent fibers in control animals. In contrast, under inflammatory conditions, AEA reduced these responses *via* CB_1 receptor activation [85]. Intrathecal application of

WIN55,212-2 *via* activation of CB_1 receptors attenuated mechanical hypersensitivity associated with peripheral inflammation induced by complete Freud's adjuvant (CFA) injection and also present after peripheral neuropathy caused by partial ligation of the sciatic nerve [86,87]. Increased AEA level in the spinal cord was reported in neuropathic pain model with chronic constriction injury (CCI) of the sciatic nerve [88]. Intrathecal AEA administration blocked carrageenan-induced thermal hyperalgesia [89] and CCI-induced mechanical allodynia *via* both CB_1 and TRPV1 receptor-dependent mechanisms [90]. Suppression of spinal AEA degradation by FAAH inhibition led to the TRPV1-mediated analgesic effect in neuropathic rats while supporting experiments indicated the lipoxygenase-mediated remodeling of AEA metabolism [91].

Clinical aspects of anandamide-mediated analgesia

Ongoing research focuses on the use of exo- and endo-cannabinoids to treat pain. Exocannabinoids, naturally occurring phytocannabinoids from the cannabis plant, and synthetic cannabinoids differentiate from endocannabinoids synthesized within the body in chemical structure and pharmacological properties upon activation of the classical cannabinoid receptors CB_1 and CB_2, reflecting their different origins. The beneficial effect of Sativex (Nabiximols), a cannabis-based pharmaceutical product containing Δ^9-tetrahydro-cannabinol (Δ^9-THC) and cannabidiol (CBD) approved for pain and spasticity treatment in patients with multiple sclerosis in 2005 in Canada, was confirmed in patients with peripheral neuropathic pain in further clinical trials [92,93]. The approval of Sativex encouraged further studies of new analgesics targeting the endocannabinoid system, modulating AEA levels and related signaling.

Clinical trials looking for AEA analgesic properties in pathological pain states and neurological disorders were supported by positive preclinical results [91,94,95]. However, the failure of these clinical trials testifies to the complexity of the AEA-induced effect, including the regulation of a wide range of physiological processes, which could underlie severe side effects. It also demonstrates the difficulty of the translation of promising results from animal models to clinical settings in humans. Several inhibitors of the AEA degradation enzyme FAAH entered clinical trials also with a focus on pain relief. These FAAH inhibitors elevated plasma AEA levels and were well tolerated [96]. However, clinical trials targeted at pain relief failed to produce analgesia in patients with osteoarthritic pain manifestation [97]. Clinical interest in this area waned when phase I of clinical trials testing BIA 10-2474 was terminated for tragic fatality in the group of volunteers receiving the highest dose [98]. Activity-based protein profiling revealed off-target BIA 10-2474 activities that may have contributed to the induced neurotoxicity [99]. However, the adverse effects of BIA 10-2474 remain unexplained [100]. The great hope of clinicians was to test peripherally restricted cannabinoid agonists based on preclinical research clearly showing the analgesic effects of peripheral cannabinoid receptor activation [2,3,81]. However, clinical trials using AZD1940 and AZD1704 have failed to produce any analgesic effect [101,102]. It is unclear if an optimal activation of CB_1 receptors to produce analgesia was achieved or if peripheral CB_1 receptor stimulation failed to inhibit nociceptive signaling [103]. A better understanding of the underlying mechanisms of AEA-induced effects and the differences between rodents and humans is essential to advance preclinical and translational research.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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