Invited Review Article

Non-opioid Analgesics and the Endocannabinoid System

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Non-steroidal anti-inflammatory drugs (NSAIDs) are known to produce antinociceptive effects mainly through peripheral COX-inhibition. Paracetamol and dipyrone are different from classical NSAIDs, because they exert weak anti-inflammatory activity; mechanisms other than peripheral COX inhibition appear to play role in their antinociceptive actions. In this review, we specified classical NSAIDs, paracetamol and dipyrone as "non-opioid analgesics" and discussed the mechanisms mediating participation of the endocannabinoid system in the antinociceptive effects of these analgesics. Non-opioid analgesics and their metabolites may activate cannabinoid receptors. In addition, several mechanisms are implicated in the elevation of endocannabinoid levels following administration of non-opioid analgesics. Of these, reduction of endocannabinoid degradation via FAAH and/or COX-2 inhibition, accumulation of arachidonic acid to endocannabinoid biosynthesis following COX inhibition, inhibition of cellular uptake of endocannabinoids directly or following inhibition of nitric oxide synthase production, and induction of endocannabinoid release are among the proposed mechanisms.

Keywords: endocannabinoids, dipyrone, NSAIDs, paracetamol

Cannabinoids are a heterogenous group of compounds, which activate the cannabinoid receptors found throughout the body. They are not only found in the cannabis plant, but also produced by the human body (endocannabinoids). In addition, there are artificially synthesized synthetic cannabinoids, who are functionally similar to 9-tetrahydrocannabinol (THC), the main psychoactive and analgesic compound found in the plant (1, 2). All of these compounds exert their effects via interaction with cannabinoid-1 (CB1) and cannabinoid-2 (CB2) receptors (3, 4). Both CB1 and CB2 receptors are G-protein-coupled receptors, primarily exhibiting Gi/o signaling mechanisms. CB1 receptors are mainly expressed in brain structures, but also in peripheral tissues; on the other hand, CB2 receptors are expressed most abundantly in immune system cells in the periphery. Many of the unwanted effects of cannabinoid receptor agonists are caused via CB1 receptor located in the central nervous system (5-6).

Endocannabinoid system is comprised of CB1 and CB2 receptors, their endogenous lipid ligands (endocannabinoids) and the enzymes involved in their biosynthesis and inactivation. Endocannabinoids are derived from arachidonic acid (AA); anandamide (arachidonyl ethanolamide, AEA) and 2-arachydonoylglycerol (2-AG) are best characterized and considered to be the main endocannabinoids (6, 7). AEA and 2-AG are produced at post-synaptic neurons with two-step processes. Phosphatidylethanolamine is converted to N-acyl-phosphatidylethanolamine (NAPE) by the enzyme N-acyltransferase (NAT); then NAPE is hydrolyzed to N-acylethanolamines, such as AEA, by a NAPE-selective phospholipase D (NAPE-PLD). On the other hand, 2-AG is synthesized from diacylglycerol (DAG) by diacylglycerol lipase (DAGL), following production of DAG from inositol phospholipids (7-9). Endocannabinoids are synthesized "on demand", released immediately, act in an autocrine or paracrine manner, and their biological actions rapidly terminate (8, 10). AEA and 2-AG are removed from the extracellular space by a cellular uptake mechanism, followed by enzymatic inactivation. Relatively little is known about 2-AG uptake, but N-arachydonoyl-phenolamine (AM-404), the metabolite of paracetamol, is known to inhibit uptake of AEA. This is important with regard to the interaction of the analgesics and the endocannabinoid system, the main concept of this review. AEA is predominantly degraded to AA and ethanolamine by fatty acid amidase hydrolyase (FAAH), whereas 2-AG is predominantly metabolized to AA and glycerol by monoacylglycerol (MAGL), and to a lesser extent by FAAH (9, 11). In addition to FAAH and MAGL, AEA and 2-AG are demonstrated to be good substrates for cytochrome p450 monoxygenases,
lipoxygenases and cyclooxygenases, mainly COX-2 (12-14). Besides FAAH and MAGL inhibition, these pathways of degradation are also important in elevation of endocannabinoid levels; since all NSAIDs inhibit COX enzymes, COX-2 inhibition may participate in the antinociceptive effects of analgesic drugs. Cannabis has been used for the management of pain for centuries; moreover, there are numerous experimental and clinical researches suggesting effectiveness of constituents in the cannabis plant, endocannabinoids and synthetic cannabinoids in different pain models (15-17), but the number of approved cannabinoid-based medicines is few. Nausea and vomiting related to chemotherapy, anorexia related to AIDS, and chronic pain and spasticity related to multiple sclerosis are the conditions cannabinoids are approved for use (6, 18), but they are generally prescribed as alternative and potential adjunctive agents in these indications. Targeting endocannabinoid system appears to be among the future therapeutic strategies (19). Inhibition of FAAH, MAGL, COX-2, the enzymes playing role in inactivation of endocannabinoids, hence enhancing local endocannabinoid levels seems one of the most attractive approaches (6, 10, 19, 20). The endocannabinoid system is the target of many therapies because it involved in a number of physiological regulation pathways, but in this review we will focus mostly on modulation on nociception.

Non-steroidal anti-inflammatory drugs (NSAIDs) exhibit moderate analgesic, anti-inflammatory and antipyretic properties; they are the most common pain relief medicines in the world. Principal mechanism of action of NSAIDs is inhibiting the activity of COX enzymes, and thereby reducing the production of prostaglandins (21). However, accumulating evidence shows that NSAIDs involve mechanisms other than COX-inhibition in their actions; interaction with nitric oxide, opioidergic, monoaminergic and cholinergic systems are among the possible mechanisms of action (22). In addition, involvement of endocannabinoid system in the analgesic effect of NSAIDs seems as an unexpected but one of the most possible mechanisms. Similar to cannabinoids/endocannabinoids (23, 24), NSAIDs inhibit pain at the peripheral, spinal and supraspinal levels (25, 26). Paracetamol and dipyrone are two different analgesic drugs, being not considered as classical NSAIDs, because they possess very little anti-inflammatory activity. Similar to classical NSAIDs, endocannabinoid system has also been shown to participate in the antinociceptive actions of paracetamol and dipyrone in recent years (27-30). In this review, we grouped classical NSAIDs, paracetamol and dipyrone together under the name of "non-opioid analgesics" and focused on the contribution of endocannabinoid system to the antinociceptive effects of these analgesic drugs.

**Link Between Classical NSAIDs and the Endocannabinoid System**

Although the primary mechanism of action of NSAIDs is inhibition of COX enzymes responsible for the production of prostaglandins, their ability to inhibit FAAH activity that is responsible for the degradation of AEA has also been shown. Augmenting endocannabinoid tone only locally by inhibiting the degradative enzymes may provide local efficacy in tissues contributing to control of nociception. In 1996, the potent anti-inflammatory drug indomethacin has been suggested to reduce FAAH activity in the mouse uterus both in vivo and in vitro (31). Then, in a series of experiments, Fowler’s research group reported that several acidic NSAIDs, including ibuprofen, ketorolac, flurbiprofen, and some of their primary metabolites inhibited FAAH (32-34). Inhibitory potencies of these NSAIDs were relatively lower, but augmented 5-10-fold as the assay pH was reduced (35-37). These are very important findings, considering lowered pH in inflamed tissues together with effectiveness of local administrations and when acidic drugs are accumulated in these tissues. Accordingly, locally administered ibuprofen and rofecoxib produced synergistic effects with AEA, and this effect was blocked by the cannabinoid CB1 receptor antagonist (38, 39). In a related study, indomethacin reduced carrageenan-induced oedema; this time CB2 receptor antagonist was effective in preventing NSAID’s action (40). In these studies, reduction of FAAH metabolism via inhibition of FAAH activity is proposed as the mechanism of action for NSAIDs-induced antinociception; however, it should be taken into consideration that FAAH inhibitory activity of NSAIDs appeared not to be potent (27, 34, 41).

Besides FAAH inhibition, another way of elevating endocannabinoid tonus via preventing their metabolism is COX-2 inhibition. The principal endocannabinoids AEA and 2-AG are good substrates for COX-2, producing prostaglandin-endomonoamides (prostamides) and prostaglandin-glycerol esters; reduction in levels of these proinflammatory and pronociceptive mediators may also contribute to their antinociceptive activity (12, 13). There is an increasing interest on differential effects of NSAIDs on COX isoenzymes. Duggan et al. (42) indicated that (R) enantiomers of ibuprofen, naproxen and flurbiprofen are potent substrate-selective inhibitors of endocannabinoid oxygenation by COX-2; these NSAIDs are considered to be inactive as COX-2 inhibitors. Similarly, ibuprofen, mefamic acid and flurbiprofen are shown to be potent inhibitors of COX-2-cyclooxygenation of 2-AG than of AA cyclooxygenation (42-44). Ibuprofen also exerted potent inhibition of AEA cyclooxygenation compared to AA oxygenation (41). Endocannabinoid preferring COX inhibitors appear to be among potential novel analgesics; simultaneous FAAH and COX inhibition also seem as an attractive target (27, 45, 46).

Increase in endocannabinoid tonus can be reached not only by decreasing their metabolism via inhibiting degradative enzymes, but also by augmenting endocannabinoid biosynthesis. Since AA is also important in endocannabinoid synthesis, COX inhibition probably provides more AA for endocannabinoid synthesis rather
than prostaglandin synthesis (22, 47). It was suggested that AA mobilization increases AEA synthesis (48). Therefore, it seems that another mechanism implicated in participation of endocannabinoids to NSAIDs’ effects is shunting of free AA from prostaglandin synthesis to endocannabinoid synthesis, although how AA participates in endocannabinoid synthesis is not known.

Regarding the involvement of the endocannabinoid system in the analgesic effects of NSAIDs, Gühring et al. (49) proposed that at the spinal level, indomethacin induced a shift of AA metabolism towards endocannabinoid synthesis; second, indomethacin lowered nitric oxide production, reducing activation of endocannabinoid transporters and thus breakdown of endocannabinoids; and third, inhibited FAAH and hence enhancing endocannabinoid levels. Spinal administration of flubiprofen and intracerebroventricular administration of celecoxib also exerted endocannabinoid-dependent antinociception (50, 51). Co-administration of ketorolac and the mixed CB1/CB2 cannabinoid receptor agonist WIN 55,212-2 produced an additive antinociceptive interaction in an inflammatory visceral pain model (16). Co-administration of a FAAH inhibitor and the COX inhibitor diclofenac also elicited a synergistic antinociceptive effect in the acetic acid model of visceral nociception (45). Contradictory findings are also worth mentioning; Silva et al. (52) reported that cannabinoid receptors do not seem to be involved in the peripheral antinociceptive mechanisms of dipyrone, diclofenac and indomethacin following intraplantar administration of the NSAIDs. Antagonism of cannabinoid receptors also did not influence diclofenac-induced antinociception when given systemically (53). In another study, neither the CB1 not the CB2 antagonist blocked the effects of the NSAIDs in chronically THC administered animals (54). Staniszek et al. (55) concluded that nimesulide inhibited spinal neuronal responses in a CB1-dependent way, but they did not detect a concomitant elevation in AEA or 2-AG levels.

**Link Between Paracetamol and the Endocannabinoid System**

Paracetamol (acetaminophen) is one of the most widely used drugs as an antipyretic and analgesic. Unlike classical NSAIDs, paracetamol does not exert any anti-inflammatory activity, whereas its analgesic activity is similar to those of NSAIDs. Inhibition of peripheral COX enzymes does not appear to be primarily responsible for the antinociceptive activity of paracetamol; but probably some central mechanisms, including the endocannabinoid system, participate in these effects (56). Inhibition of central COX, modulation of serotonergic and opioidergic systems, and inhibition of NOS are among the proposed mechanisms (57-60). In 2005, Högestatt et al. (61) reported that paracetamol, following deacetylation to p-aminophenol, is FAAH-dependently conjugated with arachidonic acid in the brain and spinal cord to form the bioactive AM-404. Then, CB1 receptors have been demonstrated to participate in both local and systemic antinociceptive effects of paracetamol (62, 63). In their detailed research, Mallet et al. (28) suggested that AM-404 indirectly activates the supraspinal CB1 receptors, which in turn reinforces the activity of descending serotonergic inhibitory pathways. The metabolite AM-404 was already known to have the ability of inhibiting uptake of AEA; moreover, it has also been shown to be a central COX inhibitor, a FAAH inhibitor, a weak CB1 activator, and a potent activator of TRPV1 (46, 61, 64-67). All of these properties of AM-404 may be related to its mediatory role in the antinociceptive activity of paracetamol. Results of another study implied that modulation of the endocannabinoid system mediate the synergistic antinociceptive effects of paracetamol combinations (68). There are also some contrary data, indicating that cannabinoid receptor antagonists did not block the effects of paracetamol, but these results were obtained in animals following chronic administration of THC or in an acute visceral pain model (54, 69). In most of these studies, pharmacological blockade or genetic deletion of cannabinoid receptors have been performed. Our group measured local endocannabinoid and N-acylethanolamide levels in the brain and spinal cord of rats to observe the interaction of paracetamol and endocannabinoids directly; we observed increase in 2-AG levels in the PAG and the RVM 12 h after paracetamol administration, but a decrease in AEA levels in the spinal cord (70). There are also researches on the contribution of the endocannabinoid system to some other effects of paracetamol. It was suggested that paracetamol exhibit dose-dependent anxiolytic effect in mice via cannabinoid CB1 receptors (71). Paracetamol was also shown to enhance social behavior and cortical cannabinoid levels in mice in a CB1-mediated way (72). On the other hand, antagonism of cannabinoid CB1 and CB2 receptors did not prevent the antipruritic effect of systemic paracetamol (73).

**Link Between Dipyrone and the Endocannabinoid System**

Dipyrone (metamizole) is another worldwide used antipyretic and analgesic drug. Unlike classical NSAIDs, but similar to paracetamol, it possesses little anti-inflammatory activity. Despite intensive research, the precise mechanism underlying the antinociceptive effect of dipyrone is still unknown. Rather than peripheral COX inhibition, it has been suspected for a long time that dipyrone elicits centrally-mediated antinociceptive action (74-76). Initially, research has focused on the concept that endogenous opioids are involved dipyrone’s antinociception. When microinjected into PAG, dipyrone exerted antinociceptive effects mediated by endogenous opioids of the RVM (77), which then triggers descending inhibition of spinal nociception (78); role of endogenous opioids in the spinal cord was also demonstrated (79). In another study, PAG-administered dipyrone induced development of tolerance in rats (80). When administered intravenously, dipyrone also caused antinociception by activating endogenous opioid system (81). Other than its interaction with endogenous opioids, dipyrone is suggested to possess antinociceptive activity by classical COX inhibition (although weak)
(82), and activation of the L-arginine-nitric oxide pathway and the subsequent KATP channel opening (83), although there are some opposite findings (84, 85).

In 2012, Rogosh et al. (29) demonstrated two unknown metabolites of dipyrone formed in the brain and spinal cord; FAAH seemed to be responsible for the formation of these metabolites, and once formed they bind weakly to cannabinoid receptors, but were modest inhibitors of COX-1 and -2. Then, it was shown that microinjection of dipyrone into PAG elicited antinociception via CB1 receptors in an inflammatory pain model (86). There are researches that suggesting mechanisms other than endocannabinoid system for the antinociceptive effects of dipyrone, but these results were obtained under non-inflammatory conditions (30, 87). However, majority of the reports point to the important role of endocannabinoid system in nociception induced by dipyrone. It is suggested that activation of CB1, but not CB2, receptors together with neuronal KATP opening is involved in the antihyperalgesic effect of dipyrone metabolites (88). These novel metabolites reduced the activity of ON-cells and enhanced the activity of OFF-cells on RVM (25). Importantly, Crunfli et al. (47) indicated that the endocannabinoid system, especially CB1 receptors, is involved in analgesia, catalepsy and hypolocomotion induced by systemic dipyrone. They hypothesized that COX and FAAH inhibition together may increase endocannabinoid availability and exhibit abovementioned effects via CB1 receptor stimulation. In accordance with these reports, a computational analysis suggested dipyrone metabolite 4-methylaminoantipyrine as a cannabinoid CB1 receptor agonist (89). In the research mentioned in the paracetamol section, we also measured local endocannabinoid levels in the brain and spinal cord of rats following systemic dipyrone administration; dipyrone exerted no action on 2-AG levels, but unexpectedly lead to a reduction in AEA levels in the RVM and spinal cord (70). In a very recent study, dipyrone, following hydrolysis to its active metabolite 4-methylaminoantipyrine, exerted a local antihyperalgesic effect partially depending on CB2 and kappa-opioid receptors (90). Apart from studies on nociception, unlike paracetamol, systemic dipyrone did not exert anxiolytic-like effects in mice (91).

Conclusion
Cannabinoids modulate nociception at the peripheral, spinal and supraspinal levels (23, 24). After activating supraspinal cannabinoid receptors, cannabinoids inhibit the presynaptic release of GABA via CB1 receptors in the lateral-ventrolateral PAG and RVM, and hence increase the postsynaptic neuron activity (92-95). Similarly, in addition to their peripheral actions, non-opioid analgesics and/or their metabolites may augment endocannabinoid levels and/or directly activate cannabinoid receptors; then may facilitate the activity of descending inhibitory pathways, and thus decrease nociceptive transmission.

We conclude that the endocannabinoid system may participate in the antinociceptive effects of non-opioid analgesics via several mechanisms:
1- Activation of cannabinoid CB1 receptors (peripheral, spinal, supraspinal) by non-opioid analgesics and/or their metabolites (29, 88, 90),
2- Increase in endocannabinoid levels,
a) inhibition of degradative enzymes,
 i) via FAAH inhibition (34, 41),
 ii) via COX-2 inhibition (27, 42, 49),
b) shift of AA metabolism towards endocannabinoid synthesis due to COX inhibition (47-49),
c) reducing activation of endocannabinoid transporters and thus endocannabinoid degradation due to inhibition of NOS production (96, 97),
d) induction of endocannabinoid release (70, 94),
e) inhibition of cellular uptake of endocannabinoids by the metabolite (paracetamol) (22, 61).
REFERENCES


FIG. 1. Possible mechanisms of action regarding to contribution of the endocannabinoid system to the antinociceptive effects of non-opioid analgesics. Non-opioid analgesic drugs and their metabolites; 1) may activate cannabinoid receptors, 2, 3) may reduce endocannabinoid degradation via FAAH and/or COX-2 inhibition, 4) may induce arachidonic acid shift to endocannabinoid biosynthesis, 5, 6) may inhibit cellular uptake directly or via inhibiting nitric oxide synthase production, and finally 7) may stimulate endocannabinoid release.