

**Back to Basics**

## Potential Analgesic Mechanisms of Acetaminophen

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Despite nearing the end of the decade of pain research, the analgesic mechanisms of one of the most widely used and popular analgesics remains uncertain. Acetaminophen (APAP) (paracetamol [PARA]) has been used clinically for over a half of a century and although clinicians seem to be comfortable with its benefits, risks, and limitations, they still remain in the dark as to precisely what is providing its pain relief. What does seem clearer is that the predominant mechanisms of APAP's analgesic effects are in the central nervous system (CNS).

Although, which central effects are largely responsible for APAP's effects on pain continue to be uncertain. Perhaps, the most accepted theory is that of APAP's positive effects on the serotonergic descending inhibitory pathways. However, interactions with opioidergic systems, eicosanoid systems, and/or nitric oxide containing pathways may be involved as well.

Furthermore, endocannabinoid signaling may play a role in APAP's activation of the serotonergic descending inhibitory pathways. A greater understanding of APAP's analgesic mechanisms may promote optimal utilization of analgesic polypharmacy.

**Key words:** Acetaminophen (APAP), paracetamol (PARA), pain, analgesia, mechanisms of action, serotonin, opioids, endocannabinoids

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**A**cetaminophen (APAP) continues to be one of the most popular analgesics available in the United States despite that the mechanisms of antinociception elicited by APAP are not yet elucidated with certainty.

Although paracetamol (PARA) appears to be the recommended international nonproprietary name of APAP, acetaminophen (or APAP) will be used in this article since the term "paracetamol" is not widely recognized in the United States. APAP was synthesized in 1878 by Morse (1) and first used clinically by von

Mering in 1887 (2). However, during the period when phenacetin was popular, its use was dormant. The studies of Brodie and Axelrod (3) led to its "rediscovery" and marketing in the 1950s in the United States as an analgesic replacement for phenacetin, which was abandoned due to its nephrotoxicity. APAP became available in the United States in 1955 and in the United Kingdom the following year. Unfounded concerns about APAP's safety delayed its widespread acceptance until the 1970s (4). APAP remains the most popular analgesic/antipyretic used in children.

## POTENTIAL MECHANISMS OF APAP-INDUCED ANALGESIA

Although suspected for many years, it is now becoming clearer that the mechanisms, which are largely responsible for APAP's analgesic effects are largely central in origin.

Crawley et al (5) studied the efficacy of systemic (oral) and intrathecal (IT) applications of APAP in preventing the development of hyperalgesia induced through the direct activation of pro-algesic spinal receptors. Spinal administration of substance P (SP, 30 nmol, IT) in rats produced a decreased thermal threshold, indicating centrally mediated hyperalgesia. Pretreatment of rats with oral APAP (300 mg/kg), but not vehicle, significantly attenuated IT SP-induced hyperalgesia. APAP given IT also produced a dose-dependent (10 – 200 µg) antinociceptive effect. In addition, oral APAP suppressed spinal PGE<sub>2</sub> release evoked by IT SP in an in vivo IT dialysis model. The ability of IT as well as oral APAP to reverse this spinally initiated hyperalgesia emphasizes the likely central action and bioavailability of the systemically delivered drug (5).

For the systemic route of delivery, the observation that APAP reversed that centrally mediated hyperalgesia is consistent with its known ability to penetrate into the brain (6) at a dose which failed to alter the acute thermal threshold (5). This emphasizes that the site of systemic drug action was within the neuraxis via mechanisms that mediate spinal sensitization (5). Similarly, the oral dose of APAP required to produce a central antihyperalgesic effect was 1,000 times the dose required when administered intrathecally. It is therefore unlikely that the spinal effect of the IT drug effect was due to redistribution of the drug into the periphery (5). Multiple mechanisms may contribute to the analgesia provided by APAP.

The results of Seo et al (7) suggest that LPS-induced hyperalgesia in the formalin second phase may be involved in the SP-sensitive neuronal pathways, in which the hyperalgesic response elicited by LPS is attenuated by APAP with supraspinal pain modulatory mechanisms.

## APAP AND THE EICOSANOID SYSTEM

Prostaglandin H<sub>2</sub> synthetase (PGHS) is the enzyme responsible for metabolism of arachidonic acid to the unstable PGH<sub>2</sub>. The 2 major forms of this enzyme are the constitutive PGHS-1 and the inducible PGHS-2. These 2 enzymes are commonly referred to

as COX-1 and COX-2 (8). However, the nomenclature PGHS is preferred because there are 2 active sites on this enzyme: a COX site and a POX site. The activity of the COX enzyme relies on its being in the oxidized form and it is suggested that APAP reduces the amount of the oxidized form by an action on the POX site (9). An alternative suggestion is that a PGHS variant (COX-3) exists in the central nervous system (CNS), and that this variant is exquisitely sensitive to APAP (10).

Rezende et al (11) concluded that although both APAP and dipyron are inhibitors of COX isoforms and thus of prostaglandin biosynthesis and were analgesic in our model, their analgesic actions were functionally and mechanistically different.

The involvement of prostaglandins (PGs) in the analgesic mechanisms of action of APAP has been proposed, taking into account the inhibition of the central cyclo-oxygenases (COX-1, COX-2, and COX-3) exerted by this drug (10,12-15), although the results are controversial in this regard (16).

Despite limitations with the belief that COX-3 may be the site of APAP action, it has been suggested that there may be varied products from the 2 distinct COX proteins with overlapping contributions to prostanoid production throughout the body (17).

Much investigation has centered on APAP's inhibition of the COX enzyme because its analgesic and antipyretic effects are similar to those of aspirin, the archetype non-steroidal anti-inflammatory drug (NSAID) (18). However, APAP does not have significant anti-inflammatory activity nor does it inhibit production of the pro-clotting TXAs. It seems reasonable to assume that although there may be some APAP effect on COX enzymes, this effect may be different from that seen with the NSAIDs (18).

The conversion of arachidonic acid to PGG<sub>2</sub> is dependent on the tyrosine-385 radical (Tyr385\*) at the COX site (19). However, generation of this radical from Tyr385 is reliant on generation of a ferryl protoporphyrin IX radical cation (Fe<sup>4+</sup>=OPP<sup>•+</sup>) at the POX site. APAP interferes with this process by acting as a reducing cosubstrate in a reaction that partially reduces Fe<sup>4+</sup>=OPP<sup>•+</sup> so that less Fe<sup>4+</sup>=OPP<sup>•+</sup> is available to be transferred to the COX site. Consequently, less Tyr385\* is available to stimulate conversion of arachidonic acid to PGG<sub>2</sub> (9,20). APAP acts with greater efficacy in environments with low peroxide tone and low arachidonic acid levels, such as what exists within the CNS (13).

Studies have shown that therapeutic concentrations of APAP have inhibited PG synthesis in brain homogenates (12) and ram seminal vesicle microsomes (21). However, these results have not been confirmed in other studies (22,23).

Graham and Scott (13) have suggested that there may be 2 important consequences of the metabolism of APAP by the peroxidase function of the COX-1 and COX-2. First, the metabolism utilizes reduced glutathione and there may be a local depletion of the glutathione. Reduced glutathione is a cofactor of many enzymes and, in particular, is a cofactor of membrane-associated PGE synthase. Consequently, the local depletion of reduced glutathione may lead to decreased production of PGE<sub>2</sub>. The second possible consequence of the metabolism of APAP is that the 2 reactive metabolites may combine directly with enzymes involved in PG synthesis and inhibit them (13). Furthermore, APAP appears to be a weak competitive inhibitor of cyclooxygenase. This is indicated by the observation that APAP can competitively inhibit the cyclooxygenase function of COX-1 under conditions in which the peroxidase function is inactive (23). The general finding at least in suboptimal conditions, is that the effect of APAP on COX-2 is weak inhibition (24-26).

It is conceivable that APAP may affect COX activity. Lee et al (27) using repeated dosing of APAP found that COX-1 gene expression was significantly down-regulated at 24 hours by ketorolac, rofecoxib, and APAP. APAP suppression of PGE<sub>2</sub> without inhibiting TXB<sub>2</sub> release, when COX-2 gene expression is up-regulated, suggests that APAP is a selective COX-2 inhibitor in vivo (27). The up-regulation of COX-2 gene and down-regulation of COX-1 gene expression suggests that APAP may result in changes in COX-derived prostanooids with reported doses (27).

Interaction with multiple other neurotransmitter systems has been proposed to explain the analgesic effects of APAP, including the serotonergic system, opioidergic, noradrenergic, cholinergic, and nitric acid (NO)-synthase systems (16,28,29). APAP may interfere with nociception associated with spinal NMDA receptor activation. This effect may involve an inhibitory action on spinal NO mechanisms (28). APAP-induced antinociception seems to derive from the synergy between peripheral, spinal, and supraspinal sites (16,30). The supraspinal components contributing to the analgesic mechanisms of APAP include opioid-like and serotonergic (31,32), with the latter (28) of much more significance (16). Although the involvement of  $\mu$ -opioid receptors in the antinociceptive

actions of APAP is still a matter of debate (16,33,34), it seems that there is at least some contribution from the opioidergic system (which may be indirect).

### **APAP AND THE OPIOIDERGIC SYSTEM**

It seems likely that at least one mechanism contributing to the analgesic mechanisms of APAP, directly or indirectly, is its effects on opioid containing pathways. Pretreatment with APAP or dipyrone (60–360 mg kg<sup>-1</sup>) reversed hyperalgesia induced by intraplantar injection of 250 microg lamda-carrageenan CG and increased nociceptive threshold in the inflamed paw above the basal level (hypoalgesia) (11). APAP, but not dipyrone, also raised nociceptive thresholds in the non-inflamed paw (11). Subcutaneous, but not local, administration of naltrexone, a specific opioid antagonist, reversed the hypoalgesia induced by APAP, but similar naltrexone treatment had no effect on dipyrone-induced analgesia (11).

Ruggieri et al (35) evaluated the possible role of the opioidergic system in the antinociceptive effect of the APAP metabolite AM404, since  $\mu$  and  $\kappa$  receptors are strongly implicated in supraspinal and spinal opioid induced analgesia. A functional relationship between the opioidergic and serotonergic systems in the pain control pathways has been suggested on the basis of behavioral studies (36). The 2 pathways are closely interconnected and can interact to modulate and produce many behavioral changes, including nociception (35,37).

Ruggieri and colleagues (35) assessed the possible implication of 5-hydroxytryptamine (5-HT) [serotonin] in the antinociceptive effect of AM404, by evaluating the influence of 5-HT<sub>1A</sub> (NAN-190), 5-HT<sub>2</sub> (ketanserin), and 5-HT<sub>3</sub> (ondansetron) receptor antagonists and studying the possible changes in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations in the frontal cortex and the pons of the rat.

Comparing the %MPE values with those obtained with APAP, it was observed that the effect of AM404 was significantly lower (about 50%) in either test (35). These data might suggest that APAP produced its effect only partially through its metabolite AM404. This finding is supported by the observation that either APAP (400 mg/kg) or AM404 (10 mg/kg) produced similar plasma AM404 concentrations (35).

Ruggieri et al (35) demonstrated the involvement of the opioid system in the antinociceptive effects of these drugs.  $\mu$ 1 and  $\kappa$  opioid receptors seem to be implicated, since the activity of AM404 was significantly

decreased by naloxonazine (a selective  $\mu$ 1 receptor antagonist) and MR2266 (a selective  $\kappa$  antagonist). Similarly, the antinociceptive activity of partially APAP was inhibited by these antagonists, suggesting that APAP also works at least in part, through the  $\mu$  and  $\kappa$  opioid receptors as previously observed (16).

APAP metabolites (e.g., AM404) seem to behave like the parent compound, activating, at least in part, the opioidergic and cannabinoid systems. The interaction between CB1 and opioid receptors may modulate many neurotransmitters, including 5-HT, glutamate, and  $\gamma$ -aminobutyric acid (35,38).

Serotonergic neurons are part of pathways which interact with and may be intertwined or blended with opioid mediated pain modulatory circuit. The interaction of opioid and serotonin systems in contributing to analgesia has been suggested to occur (39,40) via regulation of central  $\mu$  and  $\kappa$  receptors (41). Ruggieri et al (35) have shown that the block of the opioidergic system (through  $\mu$  and  $\kappa$  receptor antagonists) is able to revert the changes in 5-HT levels and serotonergic turnover induced by APAP. Therefore, the implication of both serotonergic and opioidergic systems in the activity of APAP could occur through a mechanism in which the activation of one pathway will influence the other (35).

Marek (42) demonstrated a physiological interaction between 5-HT<sub>2</sub> and  $\mu$ -opioid receptors in some cortical areas. Thus, APAP may act through both opioidergic and serotonergic systems in a synergistic manner.

### **APAP and the Serotonergic System**

The analgesic mechanism of action of APAP may involve 5-HT playing a key contributing factor as suggested by multiple authors (31,35,43): an increase in 5-HT levels in various CNS structures has been detected after APAP treatment (29). Ruggieri and colleagues (35) demonstrated a differential contribution of AM404 and APAP on the serotonergic system in their antinociceptive effects. Investigations also examined the roles of serotonergic (5-HT<sub>1A</sub>, 5-HT<sub>2</sub>, and 5-HT<sub>3</sub>) receptor subtypes in the mechanisms of APAP-induced analgesia (44).

Some type of interaction with the 5-HT system likely contributes to APAP-induced antinociception (29). In agreement with this, Bonnefont and colleagues (45) have shown that blocking spinal 5-HT<sub>1A</sub> receptors inhibits the antinociceptive action of APAP in the for-

malin test. It has been postulated that APAP-induced stimulation of 5-HT<sub>1A</sub> receptors may lead to reinforcement of bulbospinal 5-HT descending inhibitory pathways at the supraspinal level (46). However, analgesic mechanisms of APAP may depend on the impact of APAP upon both COX activities and the 5-HT system.

APAP-elicited stimulation of spinal 5-HT<sub>1A</sub> receptors, thought to be due to the reinforcement of 5-HT descending pathways, modulates pain transmission in a complex manner. Among the cellular events involved, Bonnefont and colleagues (47) described that APAP was up-regulating the expression of GH and IGF-1 receptors, which may participate in its antinociceptive activity more or less robustly.

The antinociceptive effects of APAP have been correlated with a decrease in the maximal number of cortical 5-HT<sub>2A</sub> receptors (31). Both APAP and morphine induced an antinociceptive effect in rats on day one but only APAP maintained this effect for 7 days while morphine did not (15). The number of  $\mu$ -opioid receptors decreased on days 1, 3, and 7 by a similar percentage after APAP administration (by 29, 31, and 34%, respectively), while morphine produced a progressive decrease in comparison with controls (by 37, 49, and 60%, respectively) and  $\kappa$ -opioid receptors were unaffected. Both drugs similarly decreased the 5-HT<sub>2</sub> receptor number on all days of treatment (by about 30 %) (16). Sandrini et al (16) concluded that the opioidergic and serotonergic systems are involved in different ways in the induction and maintenance of antinociception after APAP or morphine treatment.

The involvement of PGs in the mechanism of action of APAP has been proposed, taking into account the inhibition of the central cyclo-oxygenases (COX-1, COX-2 and COX-3) exerted by this drug (10,12-15), although the results are controversial in this regard. Moreover, interaction with many neurotransmitter systems, in particular with the serotonergic system, has been proposed to explain the effect of APAP: opioidergic, noradrenergic, serotonergic, cholinergic, and NO-synthase systems have been studied in this regard (28,29). Indeed, APAP-induced antinociception seems to derive from the synergy between peripheral, spinal, and supraspinal sites (30). The supraspinal components identified in the mechanism of APAP are opioid-like and serotonergic (31,32), the former contributing less than the latter (28), although the involvement of  $\mu$ -opioid receptors in the antinociceptive action of APAP

is still a matter of debate (33,34). We had previously detected an increase in serotonin concentration in the cerebral cortex and in the pons after acute APAP treatment.

The central effect of APAP is blocked by some serotonin antagonists, although the receptors have not been identified precisely (33,48-50). The depletion of brain serotonin also blocks the analgesic effect of APAP (31,51). The interaction between APAP and serotonin appears to be indirect because no binding of APAP has been detected with a variety of serotonergic receptors or on the uptake of serotonin (33,52). No significant binding or interaction with uptake sites has been found with other neurotransmitters. One possible indirect link is that APAP may interact with serotonergic pathways through a decrease in PG synthesis. Perhaps by altering CNS levels of prostanoids, APAP may affect several monoamine neuron types in the brain containing the EP3 receptor, a major receptor for PGE2 (13).

The reversal of the antinociceptive effect of systemically administered APAP by IT administration of the potent 5-HT(3) receptor antagonist tropisetron has been reported in rats subjected to the paw pressure test, suggesting that APAP action is mediated through spinal 5-HT(3) receptors (53). Unlike tropisetron, other 5-HT(3) receptor antagonists, such as ondansetron and granisetron, injected intrathecally were unable to reverse the antinociceptive effect of APAP (53). Moreover, pretreatment with spinal 5-HT(3) receptors antisense oligodeoxynucleotides (AODNs) did not reverse the APAP-induced antinociceptive effects (53). These results suggest that APAP-induced antinociceptive action involves a spinal tropisetron-sensitive receptor that is not the 5-HT(3) receptor and that remains to be identified (53).

A major hypothesis of APAP's analgesic mechanisms is APAP leading to promotion of the inhibitory activity of the descending inhibitory serotonergic pathways on spinal nociceptive processing, a theory which has been supported by different groups (29,31,51). Lesioning of the bulbospinal descending serotonergic pathways abolishes the antinociceptive action of APAP (51). The laboratory of Alain Eschaler demonstrated that different spinal 5-HT receptor subtypes are involved in the antinociceptive effect of APAP in rats (31,45) and other investigations supported this serotonergic mechanism as contributing to APAP-induced analgesia in humans

(15). However, these and other theories have not explained how the action of APAP initiates stimulation of serotonergic descending inhibitory pathways to ameliorate pain.

### **APAP and the Cannabinoid System**

The discovery of involvement of endocannabinoids on pain modulation has opened new mechanistic perspectives (54,55). Anandamide and 2-arachidonoylglycerol, 2 endogenous ligands of CB1 and CB2 receptors, mainly metabolized by the fatty acid amide hydrolase (FAAH), and the monoacylglycerol lipase, respectively, induce antinociceptive effects (56,57). Similarly, activation of this system by exogenous ligands for cannabinoid (particularly CB1) receptors induces antinociception in various acute pain tests in rodents (56,58,59) but also in several animal models of chronic pain (60). Several studies reported that cerebral injection of cannabinoids in the periaqueductal gray (PAG) or the rostroventral medulla (RVM) elicits antinociception, suggesting the modulation of descending pathways to inhibit pain processing at the spinal level (59,61,62).

Högestätt et al (63) demonstrated that APAP undergoes a two-step metabolic transformation in the brain to form N-arachidonoyl-phenolamine (AM404), which is an agonist of transient receptor potential vanilloid type 1, a ligand at selective cannabinoid subtype 1 (CB1) receptors and an inhibitor of cellular anandamide uptake, whose inhibition leads to an increase in endogenous cannabinoids (64). Cannabinoids produce antinociceptive effects that are mediated chiefly by CB1 receptors (65). Cannabinoid-induced antinociception also seems to depend, to some extent, on the release of opioid peptides into the brain (66).

APAP could be metabolized in the brain into AM404, and then inhibit the reuptake of anandamide (67), with subsequent stimulation of CB1 receptors via FAAH (63). Thus, the antinociceptive activity of APAP may rely on an interaction with the endocannabinoid system (68). Mallet et al (69) hypothesized that the interaction of APAP with the endocannabinoid system could be on the basis of the reinforcement of the serotonergic system.

Zygmunt et al (70) started from the observation of the striking structural similarity between APAP and the fatty acid amide N-arachidonoyl-phenolamine (AM404). Zygmunt and colleagues (70) have shown

that APAP, following deacetylation to its primary amine (p-aminophenol) is conjugated with arachidonic acid in the brain and spinal cord to form AM404 (via FAAH), which also catalyzes the hydrolysis of anandamide and which can also act in the reverse direction and catalyze the synthesis of anandamide from ethanolamine and arachidonic acid. Zygmunt and colleagues (70) have shown that FAAH can indeed synthesize AM404 from p-aminophenol and arachidonic acid *in vitro*, and that, in addition, no formation of AM404 is observed *in vitro* or *in vivo* in brain tissue from FAAH gene in knockout mice. Bertolini and colleagues (71) produced experimental data suggesting that the analgesic activity of APAP involves potentiation of the cannabinoid/vanilloid tone in the brain and in dorsal root ganglia.

APAP does not bind to CB<sub>1</sub> receptors but rather activates CB<sub>1</sub> receptors via an indirect pathway relying on FAAH-dependent AM404 formation and subsequent AM404 effects on anandamide transport.

Mallet et al (69) assessed the influence of FAAH inhibitors on the antinociceptive activity of APAP. Phenylmethylsulphonyl fluoride (PMSF), an FAAH inhibitor, (10 mg/kg, *s.c.*), used at a dose shown to be able to abolish the FAAH-dependent metabolism of APAP (63), significantly inhibited the antinociceptive effect of APAP in both the paw pressure and the formalin tests (69). In addition, URB597 (0.15 mg/kg, *i.p.*), an irreversible brain-penetrating FAAH inhibitor, also reversed the antinociception elicited by APAP (69).

Jayamanne et al (72) reported that systemic administration of a selective FAAH inhibitor URB597 (0.3 mg kg<sup>-1</sup>) reduced the mechanical allodynia and thermal hyperalgesia in the complete Freund's adjuvant (CFA) model of inflammatory pain. The effects of URB597 in the CFA model were dose dependent and were reduced by coadministration with the cannabinoid CB<sub>1</sub> antagonist AM251 (1 mg kg<sup>-1</sup>), or the CB<sub>2</sub> and SR144528 (1 mg kg<sup>-1</sup>). Coadministration with AM251 plus SR144528 completely reversed the effects of URB597. These findings suggest that the FAAH inhibitor URB597 produces cannabinoid CB<sub>1</sub> and CB<sub>2</sub> receptor-mediated analgesia in inflammatory pain states (72).

La Rana et al (73) investigated the effects of the endocannabinoid transport inhibitor AM404 [N-(4-hydroxyphenyl)-eicosa-5,8,11,14-tetraenamide] on rodent models of acute and persistent nociception (intraplantar formalin injection in the mouse),

neuropathic pain (sciatic nerve ligation in the rat), and inflammatory pain (complete Freund's adjuvant injection in the rat). In the formalin model, administration of AM404 (1-10 mg/kg *i.p.*) elicited dose-dependent antinociceptive effects, which were prevented by the CB<sub>1</sub> cannabinoid receptor antagonist rimonabant (SR141716A; 1 mg/kg *i.p.*) but not by the CB<sub>2</sub> antagonist SR144528 (1 mg/kg *i.p.*) or the vanilloid antagonist capsazepine (30 mg/kg *i.p.*) (73). Comparable effects were observed with UCM707 [N-(3-furylmethyl)-eicosa-5,8,11,14-tetraenamide], another anandamide transport inhibitor. In both the chronic constriction injury and complete Freud's adjuvant model, daily treatment with AM404 (1-10 mg/kg *s.c.*) for 14 days produced a dose-dependent reduction in nocifensive responses to thermal and mechanical stimuli, which was prevented by a single administration of rimonabant (1 mg/kg *i.p.*) and was accompanied by decreased expression of cyclooxygenase-2 and inducible nitric-oxide synthase in the sciatic nerve (73).

Caballero et al (74) evaluated the effect of AM404 in human T cells, discovering that AM404 is a potent inhibitor of T-cell receptor (TCR)-mediated T-cell activation. They found that AM404 specifically inhibited both IL-2 and TNF- $\alpha$  gene transcription and TNF-synthesis in CD3/CD28-stimulated Jurkat T cells in a FAAH independent way (74). AM404 inhibited both the binding to DNA and the transcriptional activity of endogenous NFAT and the transcription activity driven by the over expressed fusion protein Gal4-NFAT. However, AM404 did not affect early steps in NFAT signaling such as CD3-induced calcium mobilization and NFAT1 dephosphorylation (74).

Different studies reported the implication of the endocannabinoid system in pain modulation either at spinal or supraspinal levels (37). Notably, the stimulation of CB<sub>1</sub> receptors in PAG and RVM reduced GABA release from the presynaptic boutons of local interneurons which can reduce the GABAergic negative influence on the inhibitory descending pathways (75,76). The antinociceptive activity of APAP has been shown to depend on spinal serotonergic receptors (33,43,45,77) and that the source of spinal 5-HT comes exclusively from supraspinal centers and mainly from RVM (37). Mallet and colleagues (69) suspected that the participation of the endocannabinoid system in the effect of APAP would occur through the reinforcement of the activity of the bulbospinal seroto-

nergic pathways. Mallet et al (69) demonstrated that the antinociceptive effect induced by a CB1 receptor agonist arachidonyl-2'-chloroethylamide (ACEA) needed intact descending bulbospinal serotonergic pathways. Furthermore, ACEA-dependent recruitment of the serotonergic system involved similar spinal 5-HT receptors to those involved in the antinociceptive action of APAP and 5-HT (43,45,46,48,50), i.e., the 5-HT<sub>1A</sub> receptor in the formalin test and the 5-HT<sub>3/4</sub> receptor in the paw pressure test. This similar 5-HT-dependent mechanism and the inhibition of the effect of APAP by inactivation of CB1 receptors suggest that the endocannabinoid system is an important link between APAP and 5-HT to produce antinociception (69).

NSAIDs inhibit fatty-acid amide hydrolase FAAH, the enzyme responsible for the metabolism of anandamide, an endocannabinoid (78). Guindon et al (78) showed that the analgesic effect of the combination of NSAIDs and anandamide was synergistic. Guindon and colleagues (78) also found that paw tissue levels of anandamide, oleoylethanolamide, and palmitoylethanolamide (PEA) were significantly higher when anandamide was combined with NSAIDs and that this effect was greater with rofecoxib.

It also remains possible that AM404 mediates the antinociceptive activity of APAP through other mechanisms (69). For instance, AM404 could activate the TRPV1 receptor, whose stimulation has been shown to elicit antinociception in the PAG (61) and to be active in the tetrad tests (79). In addition, AM404 has been shown to inhibit COX activities *in vitro*, with a potency close to those of NSAIDs (63). The involvement of a central COX inhibition during the APAP-induced antinociceptive and/or antipyretic activities will have to be confirmed *in vivo* (69). The fact that AM404 is not found in blood after administration of APAP (63) might explain why APAP does not exert any significant peripheral anti-inflammatory effect (69). This could therefore further explain why APAP does not exert significant clinical anti-inflammatory effect. Other studies stated that the antinociceptive activity of APAP may rely on a decrease in spinal NO (e.g., 4), which could also be a consequence of the reinforcement of the endocannabinoid system (69,80).

The data of Mallet et al (69) together with results published by Högestätt et al (63) suggest that the activity of APAP, orally administered, would require

a multi-step process. Firstly, the drug would need to be metabolized in 2 steps to form cerebral FAAH dependent AM404. This metabolite could reinforce the activity of the endocannabinoid system through CB1 receptors, which would in turn reinforce the activity of the bulbospinal serotonergic inhibitory pathways (Fig. 1). Finally, antinociception would occur at the spinal level where activation of stimulus-dependent 5-HT receptors would block the transmission of nociceptive influx (69).

Still another theory has been advanced to help partially explain the analgesic effects of APAP. Smith (81) has proposed that it is conceivable that increased levels of AM404 (resulting from increased APAP's conversion to AM404 via FAAH) may compete with other substrates for FAAH and thus may lead to increased levels of PEA which exhibits antinociceptive activity likely via agonist effects at the peroxisome proliferators-activated receptor- $\alpha$ . Smith (81) has postulated that increased anandamide may increase PEA levels in tissues by competing with PEA for FAAH-mediated hydrolysis (if most of the FAAH is being used to synthesize AM404 from APAP and/or metabolize anandamide to arachidonic acid and ethanolamine, then it is conceivable that there may be functionally less FAAH to metabolize PEA, and thus, effectively resulting in higher levels of PEA). Smith (81) then reasoned that some of APAP's analgesic action (albeit a minor problem) may derive from the increased levels of PEA, a compound which may exhibit analgesic activity at least in part by activating the nuclear receptor peroxisome proliferator-activated receptor —  $\alpha$  (PPAR- $\alpha$ ). LoVerme et al (82) demonstrated the PPAR- $\alpha$  mediates the anti-inflammatory actions of PEA, a compound which Calignano et al (83,84) suggested may function as an endogenous regulator of nociception.

Broad-spectrum analgesia by PEA has been documented in a variety of pain models. PEA reduces pain behaviors elicited by formalin (83,85), magnesium sulfate (84), carrageenan (86,87), nerve growth factor (88), and turpentine (85,89). Moreover, PEA was found to inhibit hyperalgesia after sciatic nerve ligation, a model of neuropathic pain (90). Because PEA-induced analgesia is rapid and precedes the compound's anti-inflammatory actions, it has been suggested that PEA may function as an endogenous regulator of nociception (83).

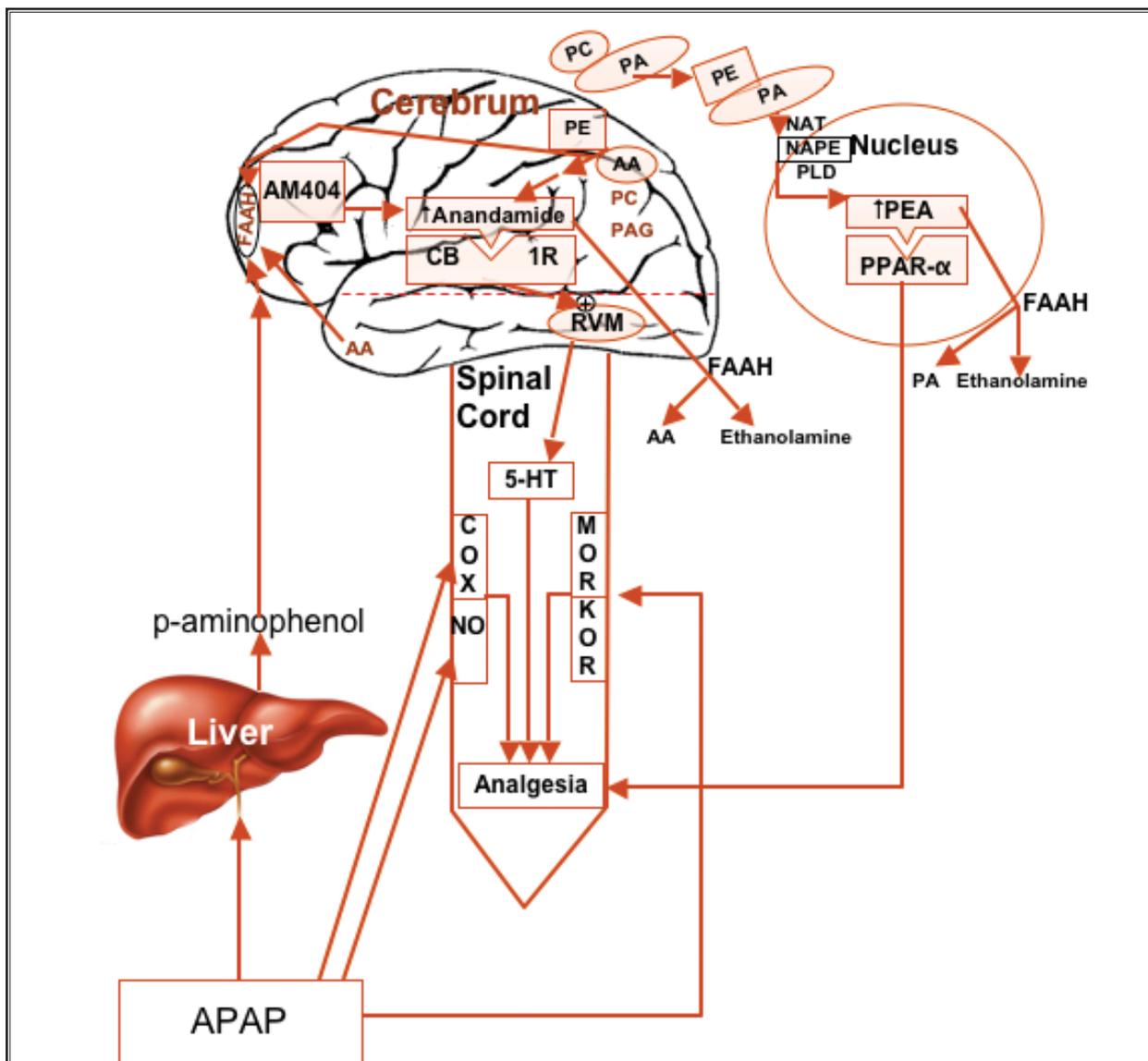


Figure 1 – Potential Analgesic Mechanisms of APAP

**Legend**

- |                                     |   |
|-------------------------------------|---|
| AA-arachidonic acid                 | NO-Nitric Oxide   |
| APAP-Acetaminophen                  | PA-Palmitic Acid  |
| CB1R-Cannabinoid 1 receptor         | PAG-Periaqueductal Grey                                 |
| COX-Cyclooxygenase                  | PC-Phosphatidylcholine                                  |
| FAAH-Fatty acid amide hydrolase     | PE-Phosphatidylethanolamine                             |
| KOR-Kappa opioid receptor           | PEA-Palmitoylethanolamine                               |
| MOR-Mu opioid receptor              | PLD-Phospholipase D                                     |
| NAPE-N-acylphosphatidylethanolamine | PPAR-α-Peroxisome proliferator-activated receptor-alpha |
| NAT-N-acyltransferase               | RVM-Rostral Ventral Medulla                             |

Fig. 1. Potential analgesic mechanisms of APAP.

## CONCLUSION

The precise analgesic actions of APAP remain uncertain. It is becoming clear that the predominant mechanisms largely responsible for APAP's analgesic activity are located in the CNS. Also, it appears that there may be multiple mechanisms (which may be in-

terlinked) which may contribute to APAP's analgesic activity. A greater understanding of how APAP provides pain relief may lead to optimal analgesia as well as improved utilization of analgesic polypharmacy.

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