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Paracetamol: Update on its Analgesic Mechanism of Action

Christophe Mallet, Alain Eschalier and
Laurence Daulhac

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Abstract

Paracetamol is the most widely used over-the-counter medication in the world. The mechanism of action of its analgesic effect was often considered as based on the mobilization of the cyclooxygenases and more recently on serotonergic pathways. A new metabolic pathway involving the generation of an active metabolite, AM404 (N-(4-Hydroxyphenyl)-5Z,8Z,11Z,14Z-eicosatetraenamide), in the brain by the fatty acid amide hydrolase (FAAH) enzyme, was recently identified. This chapter describes experimental data that have shown the involvement of this metabolic pathway in the analgesic action of paracetamol and its relationship with the cyclooxygenase and serotonergic systems. It also explains how new targets and systems, such as the cannabinoid and vanilloid systems and the calcium channel receptor Cav3.2, play a role in the action of paracetamol. Finally, it suggests how research on the mechanism of the clinically relevant effects of this long-established analgesic could lead to new therapeutic pain strategies.

Keywords: paracetamol, para-aminophenol, AM404, pain, FAAH, CB_1, TRPV1, Cav3.2, serotonin

1. Introduction

More than a century after its discovery, paracetamol (acetaminophen) is the most widely prescribed analgesic in the world. Although used as a treatment for moderate pain and fever for more than a century, the mechanisms of its analgesic action are poorly understood and are a topic of ongoing debate. This chapter presents and updates the preclinical data on the pharmacodynamics of the paracetamol. While the two main mechanisms are considered as based

on the inhibition of cyclooxygenases and/or the activation of the serotonergic system [1], we show that the endocannabinoid and vanilloid systems and the T-type calcium-channel Cav3.2 are emerging as new targets of its action *via* complex metabolic and neuronal pathways.

2. Paracetamol, a prodrug of which AM404 is the active metabolite

In 2005, Högestätt et al. [2] showed that paracetamol, following its hepatic deacetylation to *p*-aminophenol, is metabolized in the brain by the fatty acid amide hydrolase (FAAH) enzyme to form AM404 (N-(4-Hydroxyphenyl)-5Z,8Z,11Z,14Z-eicosatetraenamide) (**Figure 1**).

After administration of deuterium-labeled paracetamol in rats, they detected deuterium-labeled AM404 and *p*-aminophenol in the brain. They further showed that formation of *p*-aminophenol was present in all tissues, with highest levels in the liver and that AM404 was mainly found in the brain. The latter results were confirmed in a recent study [3].

Incubation of brain homogenate with *p*-aminophenol *in vitro* but not with paracetamol (except at high doses) leads to the formation of AM404 [2]. This is not the case if brain
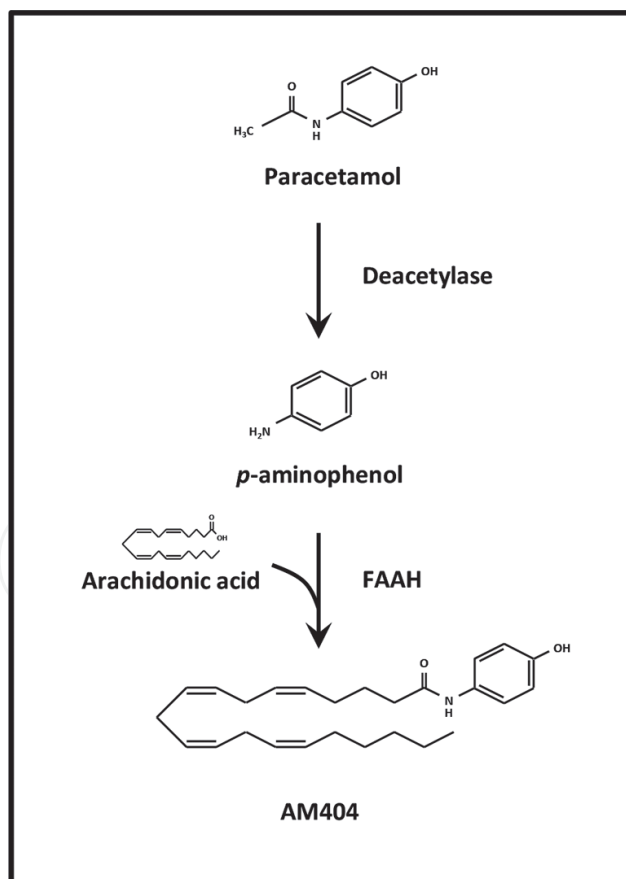

Figure 1. Metabolization of paracetamol into AM404. AM404: N-(4-hydroxyphenyl)-5Z,8Z,11Z,14Z-eicosatetraenamide. FAAH, fatty acid amide hydrolase.

homogenate is boiled, pretreated with PMSF (a broad-spectrum protease, esterase and amidase inhibitor [4]) or if brain homogenate comes from FAAH$^{-/-}$ mice. Incubation of isolated FAAH with p-aminophenol and arachidonic acid leads to the formation of AM404. *In vivo*, paracetamol does not produce AM404 in the brains of rats pretreated with PMSF or in FAAH$^{-/-}$ mice.

We speculated that this metabolic pathway was involved in its analgesic action and decided, therefore, to investigate the analgesic effect of paracetamol metabolites. Systemic administration of p-aminophenol or intracerebroventricular injection of AM404 produced an analgesic effect in animals.

We then investigated the involvement of FAAH in the action of paracetamol using mice deleted for the FAAH gene (genetic strategy) and systemic administration of PMSF or URB597, nonspecific and specific FAAH inhibitors, respectively, (pharmacological strategy) to inhibit the FAAH enzyme. Both strategies resulted in the abolition of paracetamol-induced (1) brain synthesis of AM404 and (2) analgesic action [5]. Likewise, the analgesic effect and brain formation of AM404 induced by p-aminophenol were decreased in FAAH$^{-/-}$ mice and in rats pretreated with PMSF [6].

The involvement of FAAH in the action of paracetamol was observed in different pain tests (paw pressure, von Frey, tail immersion and formalin tests) and modalities (thermal, mechanical and chemical *stimuli*) [5–7]. However, the experiments were conducted in naive animals, in a context far removed, therefore, from the clinical setting, in which paracetamol is used for pathological pain, notably nociceptive pain [8, 9]. Thus, the involvement of FAAH in the action of paracetamol was studied in a more relevant clinical context using an inflammatory mouse model submitted to thermal and mechanical *stimuli* to assess allodynia and hyperalgesia. The anti-allodynic and anti-hyperalgesic effects of paracetamol observed in this model were lost in FAAH$^{-/-}$ mice [10], which lend further weight to the involvement of the FAAH in inflammation.

Although it is now generally acknowledged that the action of paracetamol is central rather than peripheral, opinions still differ [11, 12]. FAAH is a ubiquitous enzyme [4]. Some authors detected AM404 in blood after paracetamol administration [13]. We investigated the peripheral versus central involvement of FAAH in the action of paracetamol studying its effect with an FAAH inhibitor that readily crosses the blood-brain barrier, URB597 and a peripherally restricted FAAH inhibitor, URB937 [14, 15] (**Figure 2A**).

The fact that the analgesic action of paracetamol is maintained after URB937 administration and lost after URB597 treatment [10] shows that only brain and not peripheral, FAAH is involved and thereby confirms the central action of paracetamol. As a counterproof, the peripherally restricted FAAH inhibitor URB937 was intracerebroventricularly injected and challenged with paracetamol (**Figure 2B**). A supra-spinal injection of URB937 in mice prior to paracetamol reversed its analgesic actions.

All these results show that supra-spinal FAAH is required for the desired effect of the paracetamol.
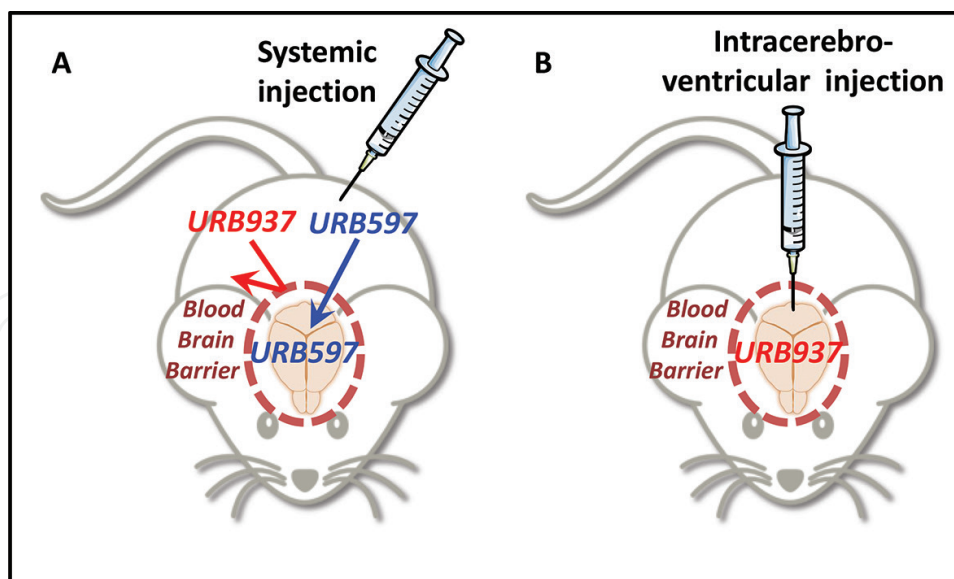
Figure 2. Pharmacological strategies to block central and/or peripheral FAAH. (A) Global or peripheral FAAH was inhibited by a systemic injection of URB597 (a brain permeant compound) or URB937 (a peripherally restricted FAAH inhibitor), respectively. (B) URB937 was supraspinally injected to specifically inhibit brain FAAH.

3. Different molecular targets of AM404

3.1. COX enzyme

The first historical hypothesis for the action of paracetamol, proposed by Flower and Vane, was the inhibition of COX [16]. In cell cultures, inhibition of COX by paracetamol was observed in different cell types, brain slices, or homogenates [16–18] with conflicting results [19]. Paracetamol seems to have only a weak inhibitory effect on prostaglandin production in cell culture, with IC_{50} values mostly around 100 μM [20]. In animals, paracetamol reduced prostaglandin in cerebrospinal fluid [21], the spinal cord [22] and the brain [23, 24]. Interestingly, AM404 was shown to be an inhibitor of COX on isolated COX-1 and COX-2 and in LPS-induced prostaglandin E_2 formation in RAW264.7 macrophages [2].

However, an orally administered analgesic dose of paracetamol (200 mg/kg) in mice did not affect brain prostaglandin E_2 (PGE_2) content, while a high intraperitoneal dose (300 mg/kg), which impairs mice locomotor activity, reduced the content of prostanoid levels in the brain (PGE_2), kidneys (PGE_2) and blood (thromboxane B_2) [7]. Paracetamol has a different pharmacological profile from that of the competitive COX inhibitor ibuprofen. In a context of noninflammatory pain, ibuprofen did not reduce pain, whereas paracetamol did, as observed in the first phase of formalin tests, tail immersion and von Frey tests in mice [7]. Altogether, these results indicate that the analgesic action of paracetamol cannot be attributed to inhibition of COX. Furthermore, the inhibitory effect of paracetamol on COX observed by some authors seems more closely related to its hypothermic/antipyretic effects than to its analgesic action [21, 23].

Further studies are needed before the involvement of COX can be fully ruled out. A study showing that PGs measured in mice after administration of 200 mg of paracetamol were not

decreased was performed with naive animals [7]. In a neuroinflammatory context such as chronic pain, in which PGs contribute to the maintenance of the process, it is possible that repeated administration of paracetamol could induce an inhibition of COX and that such a mechanism could be involved in the analgesic action of paracetamol.

3.2. CB$_1$ receptor

AM404 is able to indirectly activate the cannabinoid receptor CB$_1$ by inhibiting the degrada-tion [25] and reuptake [26, 27] of anandamide. Involvement of this receptor in the action of paracetamol was confirmed by a study showing that CB$_1$ knockout mice and rats pretreated with a specific CB$_1$ antagonist (AM251) were insensitive to paracetamol [5, 28]. Corroborating these results, we showed that the analgesic effect of p-aminophenol was also suppressed by AM251 [6]. Interestingly, it was shown in a neuropathic rat pain model that the synergic or additive antinociception of paracetamol with gabapentin, memantine, or tramadol was attenuated by pretreatment with AM251 [29]. In the same study, the intrinsic analgesic effect of gabapentine, memantine, or tramadol was not affected by CB$_1$ receptor antagonist.

The involvement of CB$_1$ receptor seems independent of the potential inhibitory effect of AM404 on cannabinoid reuptake because the overall brain content of endocannabinoids (anan-damide, 2-arachidonoylglycerol and palmitoylethanolamide) was not affected by an admin-istration of paracetamol [7] or p-aminophenol [6] in mice or in rats. In addition, paracetamol does not bind directly CB$_1$ receptors [5]. Thus, the relationship between paracetamol and CB$_1$ remains to be elucidated.

3.3. TRPV1 receptor

Subsequent studies have shown that AM404 is also a potent activator of the capsaicin recep-tor TRPV1, as reported in patch-clamp experiments [30, 31]. Interestingly, local injection of AM404 in the paw of mice resulted in pain behavior (licking and lifting of the injected paw), a behavior not found in TRPV1$^{-/-}$ mice [7].

The contribution of TRPV1 to the action of paracetamol has been explored by both genetic and pharmacological approaches to inhibit it. Results showed that a genetic inactivation of TRPV1 abolished the antinociceptive effects of paracetamol in the mouse formalin, von Frey and tail immersion tests [7]. Pharmacological blockade of TRPV1 by capsazepine in rats also suppressed the analgesic effect of paracetamol [7]. Observations made on paracetamol can be extended to p-aminophenol, since pretreatment with capsazepine in rats or administration in TRPV1$^{-/-}$ mice prevented the antinociceptive effect of p-aminophenol [6]. Further, the anal-gesic effect of the intracerebroventricular injection of AM404 was lost in TRPV1$^{-/-}$ mice [7]. In a calcium imaging experiment, human embryonic kidney (HEK) cells, which constitutively expressed FAAH, were transfected with TRPV1. AM404 induced intracellular calcium mobi-lization [30]. This response was not observed in cells pretreated with capsazepine or in cells that were not transfected with TRPV1. In agreement with the previous results, bath applica-tion of p-aminophenol also induced an increase in intracellular calcium, smaller and slower than that of AM404. The calcium increase induced by p-aminophenol was abolished in cells either pretreated with capsazepine or not transfected with TRPV1 [30]. The effect of TRPV1

was due to metabolization of *p*-aminophenol into AM404 because *p*-aminophenol-induced calcium mobilization was lost in cells pretreated with an FAAH inhibitor.

To accurately establish the location of the involvement of TRPV1 in paracetamol action, systemic administration of paracetamol was challenged with the selective blockade of TRPV1 in the brain. Injection of capsazepine into the lateral ventricle of mice abolished the antinociceptive effects of paracetamol [7]. Similarly, the antinociceptive activity of *p*-aminophenol was also lost in mice intracerebroventricularly preinjected with capsazepine [6]. Collectively, these findings identify brain TRPV1 as an important effector of paracetamol.

3.4. Cav3.2 calcium channel

Arachidonic-related compounds such as anandamide and 2-arachidonylglycerol also interact with T-type calcium channels, especially the Cav3.2 subtype, an effect which mediates their analgesic property [32]. Silencing of Cav3.2 using oligonucleotide antisense [33], knockout mice [34], or pharmacological tools [35] resulted in impairment of pain in several pain tests, thereby confirming the strong role of this calcium channel in nociception. Because AM404 is the arachidonic-related metabolite of paracetamol, the role of Cav3.2 in paracetamol action was investigated [30].

Mice with deletion of the Cav3.2$^{-/-}$ gene did not show any analgesic effect after paracetamol administration. In addition, the intracerebroventricular injection of AM404 did not induce an analgesic effect in these knockout mice.

To determine whether Cav3.2 in the brain is involved in the antinociceptive effect of paracetamol, we injected TTA-A2, a Cav3.2 blocker, intracerebroventricularly before administration of paracetamol. This treatment prevented the effect of paracetamol. Spinal involvement of Cav3.2 receptors was also studied by coadministering paracetamol with an intrathecal injection of TTA-A2. In contrast to the previous results, spinal blockade of Cav3.2 did not alter the analgesic effect of paracetamol, indicating that the antinociceptive effect of paracetamol is dependent on Cav3.2 located in the brain.

AM404 seems to have an indirect action because it only weakly inhibited Cav3.2 currents ($IC_{50} = 13.7$ μM) recorded in DRG neurons by a whole-cell patch clamp method [30]. By comparison, in the same assay, TTA-A2 had an IC_{50} of 9.0 nM. As expected, neither paracetamol nor *p*-aminophenol inhibited Cav3.2 currents.

We thus addressed the putative role of TRPV1, another calcium channel, in the mobilization of Cav3.2 in the analgesic action of paracetamol. To determine whether Cav3.2 was involved upstream or downstream of the action of TRPV1, we assessed the analgesic effect of intracerebroventricular injection of either TRPV1 agonist (capsaicin) or Cav3.2 antagonist (TTA-A2) in Cav3.2$^{-/-}$ and TRPV1$^{-/-}$ mice, respectively. Unlike the action of TTA-A2, which is maintained in TRPV1$^{-/-}$ mice, the analgesic effect of capsaicin is lost in Cav3.2$^{-/-}$ mice. These results show that brain TRPV1 activation needs Cav3.2 to mediate its action and suggest that the first target of AM404 is TRPV1.

To analyze more fully the relationship between TRPV1 and Cav3.2 channels, we performed electrophysiological recordings to study the Cav3.2 current in HEK cells stably expressing the

human Cav3.2 sequence. In these cells, the Cav3.2 current induced by depolarization was not affected by the bath application of capsaicin. However, when the cells were transfected with TRPV1, application of capsaicin suppressed the Cav3.2 current.

Altogether, these behavioral and electrophysiological findings show that Cav3.2 and TRPV1 act sequentially in concert to support the analgesic action of paracetamol [30].

4. Involvement of the serotonergic system

The involvement of the serotonergic system in the action of paracetamol was first described by Tjolsen et al. [37] in 1991 and by Pini et al. [38] in 1996. They demonstrated that the analgesic effect of paracetamol was reduced after lesion of the serotonergic bulbospinal pathway by 5,6-dihydroxytryptamine or total depletion of the central serotonin (5-HT) synthesis by p-chlorophenylalanine. These results were confirmed by another team using 5,7-dihydroxytryptamine [39]. Studies showing that paracetamol did not bind serotonin receptors [38, 40] prompted investigation of the mobilization of the serotonin neurotransmitter. The results showed that paracetamol increased in a dose-dependent manner the tissue concentrations of 5-HT in the cortex, hypothalamus, striatum, hippocampus and brainstem [38, 41].

Later studies showed that the spinal role of 5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C} and 5-HT_{7} receptor subtypes of serotonin receptors was involved in the action of paracetamol [39, 42–48]. However, investigations of the involvement of 5-HT_3 receptors yielded conflicting results in both animals [36, 42, 43, 47, 49, 50] and humans [51–54]. Interestingly, some of these studies showed that tropisetron, a nonspecific 5-HT_3 receptor antagonist, blocked the analgesic effect of paracetamol. Libert et al. [36] reported that the inhibitory effect of tropisetron on the action of paracetamol was not mediated by 5-HT_3 receptor because (1) other 5-HT_3 antagonists (granisetron and ondansetron) or (2) antisense oligodeoxynucleotides directed against 5-HT_3 receptors did not reverse the paracetamol-induced antinociceptive effect, which suggests the involvement of a spinal tropisetron-sensitive receptor that is not the 5-HT_3 receptor. More work is needed to identify this spinal receptor.

These results should be treated with caution. Serotonin receptor subtypes are differently involved in paracetamol action, depending on the nature of the stimulus. For example, spinal 5-HT_{1A} is involved in the analgesic action of paracetamol assessed in the formalin test (chemical stimulus) [44] but not in the paw pressure test (mechanical stimulus) [47]. This discrepancy could be explained by the differential efficacy and power of serotonin itself relative to the noxious tests [55]. In addition, the analgesic action of spinal-administered serotonin, like that of paracetamol, is suppressed in the formalin test [44, 45] and conserved in the paw pressure test [45, 56] following the inhibition of spinal 5-HT_{1A} receptors.

Like paracetamol, p-aminophenol elicited antinociception through the serotonergic bulbospinal pathway because its effect was reversed after lesion of the pathway by 5,7-dihydroxytryptamine [6]. In addition, spinal pretreatment of rats with WAY-100,635, a 5-HT_{1A} receptor antagonist and tropisetron, a nonspecific $5\text{-HT}_{3/4}$ receptor antagonist, reduced the analgesic effect of p-aminophenol in the formalin test and the paw pressure test, respectively [6].

In light of evidence showing that paracetamol and *p*-aminophenol involved CB_1 receptors [5], we investigated the serotonergic descending bulbospinal pathways and spinal 5-HT receptors in the antinociceptive effect of arachidonyl-2′-chloroethylamide (ACEA), a CB_1 receptor agonist. Our results showed that ACEA needed intact descending bulbospinal serotonergic pathways. Elsewhere, it was shown that the antinociceptive action of ACEA was suppressed by intrathecal injection of WAY-100,635 and tropisetron in the formalin test and the paw pressure test, respectively [5]. The similar serotonergic profiles of ACEA and paracetamol suggest that CB_1 receptor is an important link between paracetamol and serotonin in the production of antinociception.

5. New strategies to alleviate pain: pharmacological vectorization to target brain TRPV1 receptors

A high-concentration of capsaicin, an 8% patch (Qutenza®) is used clinically in Europe and the USA to alleviate neuropathic pain. It has been suggested that its action is due to defunctionalization of peripheral TRPV1 [57]. A systemic use of TRPV1 activators is to be avoided
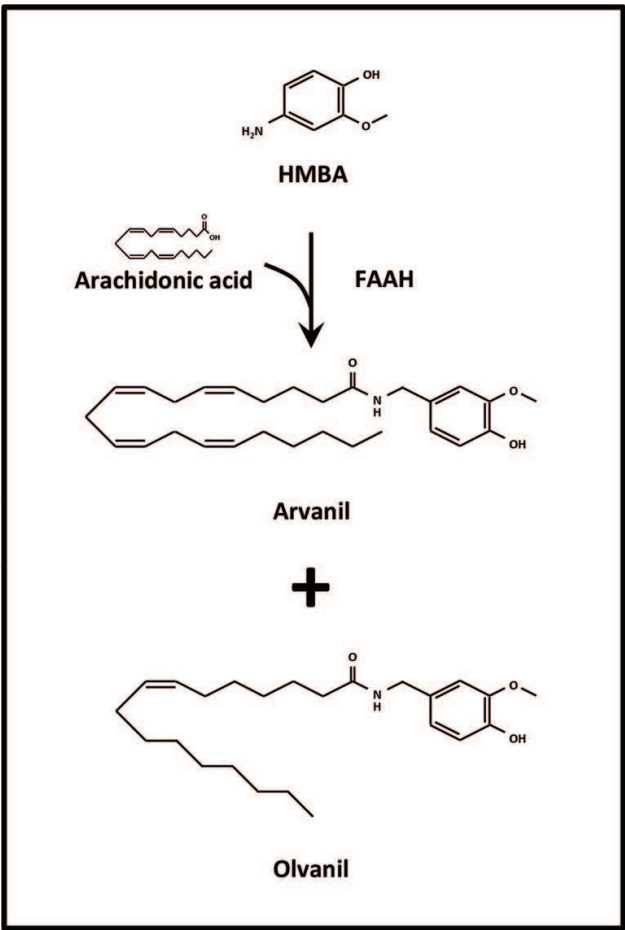

Figure 3. FAAH-dependent formation of arvanil and olvanil from HMBA.

because of their high toxicity, which entails the risk of, notably, pulmonary and cardiovascular adverse effects [58–60]. Metabolites of capsaicin could be mutagenic at very high doses as well [61]. On the basis of the study of the mechanism of action of paracetamol, we propose that brain TRPV1 should be specifically targeted for the pharmacological management of pain. New substrates of FAAH, analogs of paracetamol or p-aminophenol, can be synthesized with the idea that the arachidonic acid-conjugated metabolites would be a potent TRPV1 activators.

To validate this strategy, we studied, with E.D. Högestätt and P.M. Zygmunt, 4-hydroxy-3-methoxybenzylamine (HMBA), a primary amine analog of p-aminophenol. HMBA produced arvanil and olvanil *in vitro* in brain homogenates and *in vivo* in mouse brain [6] (**Figure 3**).

Administered in mice or in rats, it had an analgesic effect. Both the formation of arvanil and olvanil and the analgesic effect induced by HMBA were FAAH-dependent. These two effects were lower in FAAH$^{-/-}$ mice than in their FAAH$^{+/+}$ littermates. Arvanil and olvanil are potent TRPV1 activators [6, 62]. This mechanism of action contributed to the action of HMBA because, like that of paracetamol and p-aminophenol, its analgesic effect was suppressed after a genetic (TRPV1$^{-/-}$ mice) or pharmacological (rats pretreated with capsazepine) blockade of TRPV1. Finally, as with paracetamol or p-aminophenol, intracerebro-ventricular injection of the TRPV1 blocker capsazepine prevented the antinociceptive effect of HMBA [6].

Taken together, these data provide evidence of concept for the use of a pharmacological vectorization strategy aimed specifically at activating supraspinal TRPV1 to alleviate pain.

6. Conclusion

All these recent findings prompt us to propose a novel view of paracetamol as a prodrug that needs to overcome a two-step metabolism to form AM404, its active metabolite, which mediates the analgesic effect via different supra-spinal targets to activate the bulbospinal serotonergic pathways (**Figure 4**).

Interestingly, the involvement of the FAAH metabolic pathway and cannabinoid system is specifically related to their antinociceptive action and not to their hypothermic/antipyretic action [63, 64].

Several other concepts of the mechanism of action of paracetamol have been forwarded, including the involvement of the opioid [13, 65–68], adrenergic [69–71] and cholinergic [72, 73] systems and that of nitric oxide synthetase [74–77], adenosine receptors [48, 78, 79] and calcium channel TRPA1 [80]. However, other studies have yielded conflicting findings notably concerning the opioid [40, 81, 82], adrenergic [37, 71, 83] and cholinergic [84] systems.

The huge number of putative targets for the action of paracetamol and the complex relationship between all the different neurological systems complicate the study of the molecular mechanism of its analgesic action. The relationship between the putative targets needs further

investigation to provide an overall view of the action of paracetamol. The understanding of the neurological and molecular actions of clinically used analgesics such as paracetamol could pave the way for the discovery of new analgesic compounds.
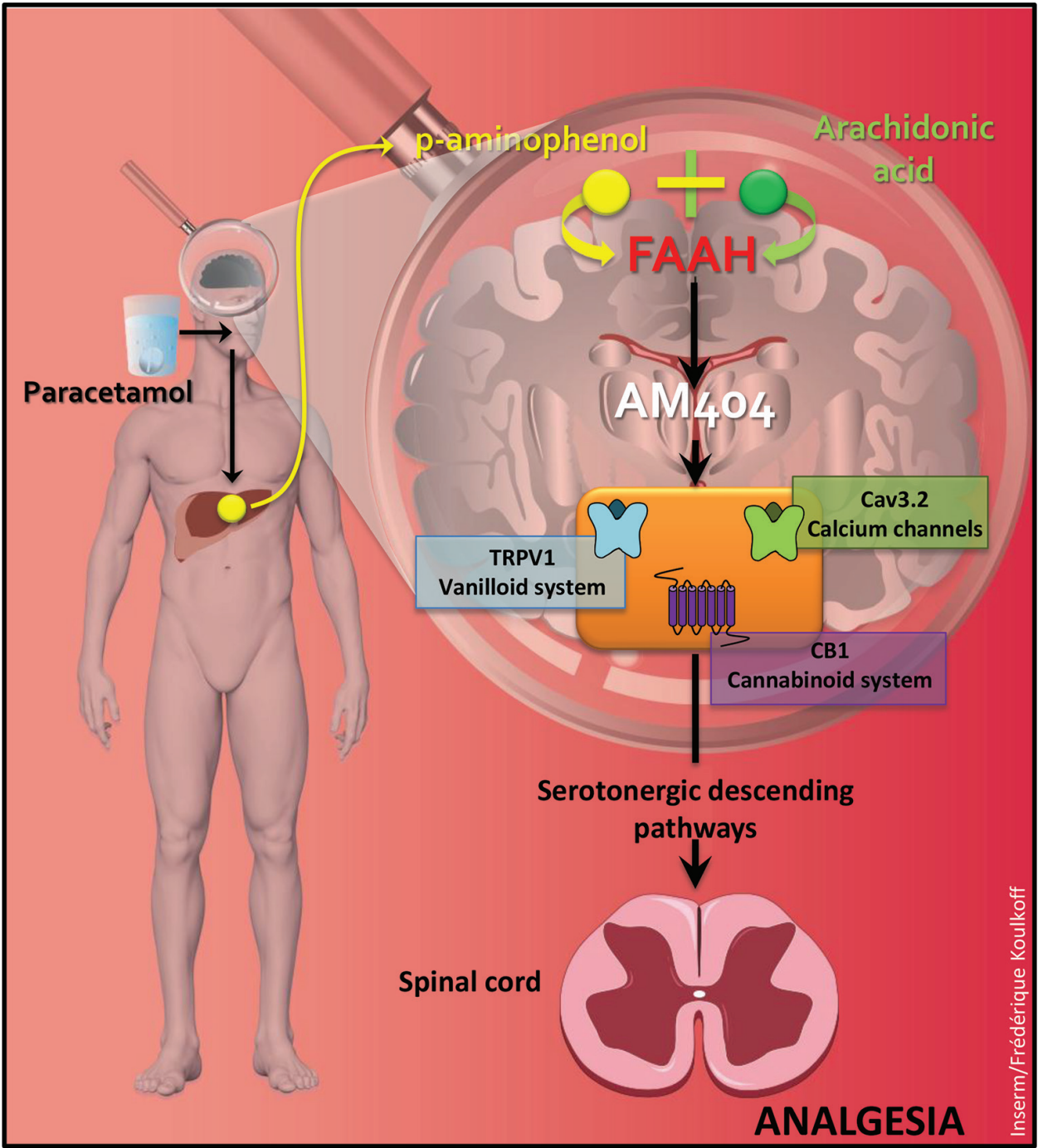

Figure 4. Proposed sequential mechanisms for the antinociceptive effect of paracetamol. (1) Deacetylation of paracetamol in *p*-aminophenol in the liver. (2) FAAH-dependent metabolism of *p*-aminophenol into AM404 in the brain. (3) Direct and/or indirect involvement of supra-spinal CB$_1$ receptors by this metabolite. (4) Reinforcement of the serotonergic bulbospinal pathways and (5) Involvement of spinal pain-suppressing serotonergic receptors. © Frédérique Koulkoff/ Inserm from Mallet/UMR 1107/Neuro-Dol Inserm.

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References

[1] C. Mallet, A. Eschalier, Pharmacology and mechanism of action of acetaminophen, in: Pharmacology of Pain, International Association for the Study of Pain, Seattle, (2010). pp. 65–86.

[2] E.D. Högestätt, B.A. Jonsson, A. Ermund, D.A. Andersson, H. Bjork, J.P. Alexander, B.F. Cravatt, A.I. Basbaum, P.M. Zygmunt, Conversion of acetaminophen to the bioactive N-acylphenolamine AM404 via fatty acid amide hydrolase-dependent arachidonic acid conjugation in the nervous system, J Biol Chem. 280 (2005) 31405–31412.

[3] S. Muramatsu, S. Shiraishi, K. Miyano, Y. Sudo, A. Toda, M. Mogi, M. Hara, A. Yokoyama, Y. Kawasaki, M. Taniguchi, Y. Uezono, Metabolism of AM404 from acetaminophen at human therapeutic dosages in the rat brain, Anesth Pain Med. 6 (2016) e32873. doi:10.5812/aapm.32873.

[4] D.G. Deutsch, N. Ueda, S. Yamamoto, The fatty acid amide hydrolase (FAAH), Prostaglandins Leukot Essent Fatty Acids. 66 (2002) 201–210.

[5] C. Mallet, L. Daulhac, J. Bonnefont, C. Ledent, M. Etienne, E. Chapuy, F. Libert, A. Eschalier, Endocannabinoid and serotonergic systems are needed for acetaminophen-induced analgesia, Pain. 139 (2008) 190–200.

[6] D.A. Barrière, C. Mallet, A. Blomgren, C. Simonsen, L. Daulhac, F. Libert, E. Chapuy, M. Etienne, E.D. Högestätt, P.M. Zygmunt, A. Eschalier, Fatty acid amide hydrolase-dependent generation of antinociceptive drug metabolites acting on TRPV1 in the brain, PLoS One. 8 (2013) e70690. doi:10.1371/journal.pone.0070690.

[7] C. Mallet, D.A. Barriere, A. Ermund, B.A. Jonsson, A. Eschalier, P.M. Zygmunt, E.D. Hogestatt, TRPV1 in brain is involved in acetaminophen-induced antinociception, PLoS One. 5 (2010). http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20862299.

[8] R.D. Altman, J.R. Zinsenheim, A.R. Temple, J.E. Schweinle, Three-month efficacy and safety of acetaminophen extended-release for osteoarthritis pain of the hip or knee:

a randomized, double-blind, placebo-controlled study, Osteoarthr Cartil. 15 (2007) 454–461. doi:10.1016/j.joca.2006.10.008.

[9]   A. Wegman, D. van der Windt, M. van Tulder, W. Stalman, T. de Vries, Nonsteroidal antiinflammatory drugs or acetaminophen for osteoarthritis of the hip or knee? A systematic review of evidence and guidelines, J Rheumatol. 31 (2004) 344–354.

[10] R. Dalmann, L. Daulhac, M. Antri, A. Eschalier, C. Mallet, Supra-spinal FAAH is required for the analgesic action of paracetamol in an inflammatory context, Neuropharmacology. 91 (2015) 63–70. doi:10.1016/j.neuropharm.2014.11.006.

[11] M. Dani, J. Guindon, C. Lambert, P. Beaulieu, The local antinociceptive effects of paracetamol in neuropathic pain are mediated by cannabinoid receptors, Eur J Pharmacol. (2007). http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db= PubMed&dopt=Citation&list_uids=17651722.

[12] S.H. Ferreira, B.B. Lorenzetti, F.M. Correa, Blockade of central and peripheral generation of prostaglandins explains the antialgic effect of aspirin like drugs, Pol J Pharmacol Pharm. 30 (1978) 133–140.

[13] V. Ruggieri, G. Vitale, L.A. Pini, M. Sandrini, Differential involvement of opioidergic and serotonergic systems in the antinociceptive activity of N-arachidonoyl-phenolamine (AM404) in the rat: comparison with paracetamol, Naunyn Schmiedebergs Arch Pharmacol. 377 (2008) 219–229.

[14] J.R. Clapper, G. Moreno-Sanz, R. Russo, A. Guijarro, F. Vacondio, A. Duranti, A. Tontini, S. Sanchini, N.R. Sciolino, J.M. Spradley, A.G. Hohmann, A. Calignano, M. Mor, G. Tarzia, D. Piomelli, Anandamide suppresses pain initiation through a peripheral endocannabinoid mechanism, Nat Neurosci. 13 (2010) 1265–1270. doi:10.1038/nn.2632.

[15] G. Moreno-Sanz, B. Barrera, A. Guijarro, I. d'Elia, J.A. Otero, A.I. Alvarez, T. Bandiera, G. Merino, D. Piomelli, The ABC membrane transporter ABCG2 prevents access of FAAH inhibitor URB937 to the central nervous system, Pharmacol Res. 64 (2011) 359–363. doi:10.1016/j.phrs.2011.07.001.

[16] R.J. Flower, J.R. Vane, Inhibition of prostaglandin synthetase in brain explains the antipyretic activity of paracetamol (4-acetamidophenol), Nature. 240 (1972) 410–411.

[17] A. Greco, M.A. Ajmone-Cat, A. Nicolini, M.G. Sciulli, L. Minghetti, Paracetamol effectively reduces prostaglandin E2 synthesis in brain macrophages by inhibiting enzymatic activity of cyclooxygenase but not phospholipase and prostaglandin E synthase, J Neurosci Res. 71 (2003) 844–852.

[18] M.G. Sciulli, F. Seta, S. Tacconelli, M.L. Capone, E. Ricciotti, G. Pistritto, P. Patrignani, Effects of acetaminophen on constitutive and inducible prostanoid biosynthesis in human blood cells, Br J Pharmacol. 138 (2003) 634–641.

[19] T.A. Swierkosz, L. Jordan, M. McBride, K. McGough, J. Devlin, R.M. Botting, Actions of paracetamol on cyclooxygenases in tissue and cell homogenates of mouse and rabbit, Med Sci Monit. 8 (2002) BR496–BR503.

[20] G.G. Graham, R.O. Day, M.K. Milligan, J.B. Ziegler, A.J. Kettle, Current concepts of the actions of paracetamol (acetaminophen) and NSAIDs, Inflammopharmacology. 7 (1999) 255–263. doi:10.1007/s10787-999-0008-x.

[21] W. Feldberg, K.P. Gupta, Pyrogen fever and prostaglandin-like activity in cerebrospinal fluid, J Physiol. 228 (1973) 41–53.

[22] U.S. Muth-Selbach, I. Tegeder, K. Brune, G. Geisslinger, Acetaminophen inhibits spinal prostaglandin E2 release after peripheral noxious stimulation, Anesthesiology. 91 (1999) 231–239.

[23] S.S. Ayoub, R.M. Botting, S. Goorha, P.R. Colville-Nash, D.A. Willoughby, L.R. Ballou, Acetaminophen-induced hypothermia in mice is mediated by a prostaglandin endoperoxide synthase 1 gene-derived protein, Proc Natl Acad Sci USA. 101 (2004) 11165–11169.

[24] S.S. Ayoub, P.R. Colville-Nash, D.A. Willoughby, R.M. Botting, The involvement of a cyclooxygenase 1 gene-derived protein in the antinociceptive action of paracetamol in mice, Eur J Pharmacol. 538 (2006) 57–65.

[25] S.T. Glaser, N.A. Abumrad, F. Fatade, M. Kaczocha, K.M. Studholme, D.G. Deutsch, Evidence against the presence of an anandamide transporter, Proc Natl Acad Sci USA. 100 (2003) 4269–4274.

[26] M. Beltramo, N. Stella, A. Calignano, S.Y. Lin, A. Makriyannis, D. Piomelli, Functional role of high-affinity anandamide transport, as revealed by selective inhibition, Science. 277 (1997) 1094–1097.

[27] A. Giuffrida, M. Beltramo, D. Piomelli, Mechanisms of endocannabinoid inactivation: biochemistry and pharmacology, J Pharmacol Exp Ther. 298 (2001) 7–14.

[28] A. Ottani, S. Leone, M. Sandrini, A. Ferrari, A. Bertolini, The analgesic activity of paracetamol is prevented by the blockade of cannabinoid CB1 receptors, Eur J Pharmacol. 531 (2006) 280–281.

[29] A.T. Hama, J. Sagen, Cannabinoid receptor-mediated antinociception with acetaminophen drug combinations in rats with neuropathic spinal cord injury pain, Neuropharmacology. 58 (2010) 758–766. doi:10.1016/j.neuropharm.2009.12.010.

[30] N. Kerckhove, C. Mallet, A. François, M. Boudes, J. Chemin, T. Voets, E. Bourinet, A. Alloui, A. Eschalier, Ca(v)3.2 calcium channels: the key protagonist in the supraspinal effect of paracetamol, Pain. 155 (2014) 764–772. doi:10.1016/j.pain.2014.01.015.

[31] P.M. Zygmunt, H. Chuang, P. Movahed, D. Julius, E.D. Hogestatt, The anandamide transport inhibitor AM404 activates vanilloid receptors, Eur J Pharmacol. 396 (2000) 39–42.

[32] G. Barbara, A. Alloui, J. Nargeot, P. Lory, A. Eschalier, E. Bourinet, J. Chemin, T-type calcium channel inhibition underlies the analgesic effects of the endogenous lipoamino acids, J Neurosci. 29 (2009) 13106–13114.

[33] E. Bourinet, A. Alloui, A. Monteil, C. Barrere, B. Couette, O. Poirot, A. Pages, J. McRory, T.P. Snutch, A. Eschalier, J. Nargeot, Silencing of the Cav3.2 T-type calcium channel gene in sensory neurons demonstrates its major role in nociception, Embo J. 24 (2005) 315–324.

[34] S. Choi, H.S. Na, J. Kim, J. Lee, S. Lee, D. Kim, J. Park, C.C. Chen, K.P. Campbell, H.S. Shin, Attenuated pain responses in mice lacking Ca(V)3.2 T-type channels, Genes Brain Behav. 6 (2007) 425–431. doi:10.1111/j.1601-183X.2006.00268.x.

[35] A. Francois, N. Kerckhove, M. Meleine, A. Alloui, C. Barrere, A. Gelot, V.N. Uebele, J.J. Renger, A. Eschalier, D. Ardid, E. Bourinet, State-dependent properties of a new T-type calcium channel blocker enhance Ca(V)3.2 selectivity and support analgesic effects, Pain. 154 (2013) 283–293. doi:10.1016/j.pain.2012.10.023.

[36] F. Libert, J. Bonnefont, E. Bourinet, E. Doucet, A. Alloui, M. Hamon, J. Nargeot, A. Eschalier, Acetaminophen: a central analgesic drug that involves a spinal tropisetron-sensitive, non-5-HT(3) receptor-mediated effect, Mol Pharmacol. 66 (2004) 728–734.

[37] A. Tjolsen, A. Lund, K. Hole, Antinociceptive effect of paracetamol in rats is partly dependent on spinal serotonergic systems, Eur J Pharmacol. 193 (1991) 193–201.

[38] L.A. Pini, M. Sandrini, G. Vitale, The antinociceptive action of paracetamol is associated with changes in the serotonergic system in the rat brain, Eur J Pharmacol. 308 (1996) 31–40.

[39] A. Dogrul, M. Seyrek, E.O. Akgul, T. Cayci, S. Kahraman, H. Bolay, Systemic paracetamol-induced analgesic and antihyperalgesic effects through activation of descending serotonergic pathways involving spinal 5-HT(7) receptors, Eur J Pharmacol. (2011). doi:10.1016/j.ejphar.2011.12.016.

[40] T. Pelissier, A. Alloui, F. Caussade, C. Dubray, A. Cloarec, J. Lavarenne, A. Eschalier, Paracetamol exerts a spinal antinociceptive effect involving an indirect interaction with 5-hydroxytryptamine3 receptors: *in vivo* and *in vitro* evidence, J Pharmacol Exp Ther. 278 (1996) 8–14.

[41] J.P. Courade, F. Caussade, K. Martin, D. Besse, C. Delchambre, N. Hanoun, M. Hamon, A. Eschalier, A. Cloarec, Effects of acetaminophen on monoaminergic systems in the rat central nervous system, Naunyn Schmiedebergs Arch Pharmacol. 364 (2001) 534–537.

[42] A. Alloui, T. Pelissier, C. Dubray, J. Lavarenne, A. Eschalier, Tropisetron inhibits the antinociceptive effect of intrathecally administered paracetamol and serotonin, Fundam Clin Pharmacol. 10 (1996) 406–407.

[43] A. Alloui, C. Chassaing, J. Schmidt, D. Ardid, C. Dubray, A. Cloarec, A. Eschalier, Paracetamol exerts a spinal, tropisetron-reversible, antinociceptive effect in an inflammatory pain model in rats, Eur J Pharmacol. 443 (2002) 71–77.

[44] J. Bonnefont, A. Alloui, E. Chapuy, E. Clottes, A. Eschalier, Orally administered paracetamol does not act locally in the rat formalin test: evidence for a supraspinal, serotonin-dependent antinociceptive mechanism, Anesthesiology. 99 (2003) 976–981.

[45] J. Bonnefont, E. Chapuy, E. Clottes, A. Alloui, A. Eschalier, Spinal 5-HT1A receptors differentially influence nociceptive processing according to the nature of the noxious stimulus in rats: effect of WAY-100635 on the antinociceptive activities of paracetamol, venlafaxine and 5-HT, Pain. 114 (2005) 482–490.

[46] J. Bonnefont, L. Daulhac, M. Etienne, E. Chapuy, C. Mallet, L. Ouchchane, C. Deval, J.P. Courade, M. Ferrara, A. Eschalier, E. Clottes, Acetaminophen recruits spinal p42/p44 MAPKs and GH/IGF-1 receptors to produce analgesia via the serotonergic system, Mol Pharmacol. 71 (2007) 407–415.

[47] J.P. Courade, C. Chassaing, L. Bardin, A. Alloui, A. Eschalier, 5-HT receptor subtypes involved in the spinal antinociceptive effect of acetaminophen in rats, Eur J Pharmacol. 432 (2001) 1–7.

[48] J. Liu, A.R. Reid, J. Sawynok, Antinociception by systemically-administered acetaminophen (paracetamol) involves spinal serotonin 5-HT7 and adenosine A1 receptors, as well as peripheral adenosine A1 receptors, Neurosci Lett. 536 (2013) 64–68. doi:10.1016/j.neulet.2012.12.052.

[49] P. Girard, Y. Pansart, M.C. Coppe, B. Niedergang, J.M. Gillardin, Modulation of paracetamol and nefopam antinociception by serotonin 5-HT(3) receptor antagonists in mice, Pharmacology. 83 (2009) 243–246.

[50] V. Minville, O. Fourcade, J.X. Mazoit, J.P. Girolami, I. Tack, Ondansetron does not block paracetamol-induced analgesia in a mouse model of fracture pain, Br J Anaesth. 106 (2011) 112–118.

[51] R. Jokela, J. Ahonen, E. Seitsonen, P. Marjakangas, K. Korttila, The influence of ondansetron on the analgesic effect of acetaminophen after laparoscopic hysterectomy, Clin Pharmacol Ther. 87 (2010) 672–678. doi:10.1038/clpt.2009.281.

[52] G. Pickering, M.A. Loriot, F. Libert, A. Eschalier, P. Beaune, C. Dubray, Analgesic effect of acetaminophen in humans: first evidence of a central serotonergic mechanism, Clin Pharmacol Ther. 79 (2006) 371–378.

[53] L. Ramirez, J. Cros, B. Marin, P. Boulogne, A. Bergeron, G.E. de Lafont, F. Renon-Carron, M.A. de Vinzelles, V. Guigonis, N. Nathan, P. Beaulieu, Analgesic interaction between ondansetron and acetaminophen after tonsillectomy in children: the Paratron randomized, controlled trial, Eur J Pain. 19 (2015) 661–668. doi:10.1002/ejp.587.

[54] E. Tiippana, K. Hamunen, V. Kontinen, E. Kalso, The effect of paracetamol and tropisetron on pain: experimental studies and a review of published data, Basic Clin Pharmacol Toxicol. 112 (2013) 124–131. doi:10.1111/j.1742-7843.2012.00935.x.

[55] L. Bardin, M. Bardin, J. Lavarenne, A. Eschalier, Effect of intrathecal serotonin on nociception in rats: influence of the pain test used, Exp Brain Res. 113 (1997) 81–87.

[56] L. Bardin, J. Lavarenne, A. Eschalier, Serotonin receptor subtypes involved in the spinal antinociceptive effect of 5-HT in rats, Pain. 86 (2000) 11–18.

[57] P. Anand, K. Bley, Topical capsaicin for pain management: therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8% patch, Br J Anaesth. 107 (2011) 490–502. doi:10.1093/bja/aer260.

[58] W. Johnson, Final report on the safety assessment of capsicum annuum extract, capsicum annuum fruit extract, capsicum annuum resin, capsicum annuum fruit powder, *Capsicum frutescens* fruit, capsicum frutescens fruit extract, capsicum frutescens resin and capsaicin, Int J Toxicol. 26 Suppl 1 (2007) 3–106. doi:10.1080/10915810601163939.

[59] H. Knotkova, M. Pappagallo, A. Szallasi, Capsaicin (TRPV1 Agonist) therapy for pain relief: farewell or revival?, Clin J Pain. 24 (2008) 142–154.

[60] A. Patapoutian, S. Tate, C.J. Woolf, Transient receptor potential channels: targeting pain at the source, Nat Rev Drug Discov. 8 (2009) 55–68.

[61] Y.J. Surh, S.S. Lee, Capsaicin in hot chili pepper: carcinogen, co-carcinogen or anticarcinogen? Food Chem Toxicol. 34 (1996) 313–316.

[62] L. De Petrocellis, T. Bisogno, J.B. Davis, R.G. Pertwee, V. Di Marzo, Overlap between the ligand recognition properties of the anandamide transporter and the VR1 vanilloid receptor: inhibitors of anandamide uptake with negligible capsaicin-like activity, FEBS Lett. 483 (2000) 52–56.

[63] S.S. Ayoub, G. Pryce, M.P. Seed, C. Bolton, R.J. Flower, D. Baker, Paracetamol-induced hypothermia is independent of cannabinoids and transient receptor potential vanilloid-1 and is not mediated by AM404, Drug Metab Dispos. 39 (2011) 1689–1695. doi:10.1124/dmd.111.038638.

[64] G. Corley, S.M. Rawls, Opioid, cannabinoid CB1 and NOP receptors do not mediate APAP-induced hypothermia in rats, Pharmacol Biochem Behav. 92 (2009) 503–507. doi:10.1016/j.pbb.2009.01.020.

[65] M. Bujalska, Effect of nonselective and selective opioid receptors antagonists on antinociceptive action of acetaminophen [part III], Pol J Pharmacol. 56 (2004) 539–545.

[66] L.A. Pini, G. Vitale, A. Ottani, M. Sandrini, Naloxone-reversible antinociception by paracetamol in the rat, J Pharmacol Exp Ther. 280 (1997) 934–940.

[67] R.B. Raffa, D.J. Stone, R.J. Tallarida, Discovery of "self-synergistic" spinal/supraspinal antinociception produced by acetaminophen (paracetamol), J Pharmacol Exp Ther. 295 (2000) 291–294.

[68] M. Sandrini, G. Vitale, L.A. Pini, G. Lopetuso, P. Romualdi, S. Candeletti, Nociceptin/orphanin FQ prevents the antinociceptive action of paracetamol on the rat hot plate test, Eur J Pharmacol. 507 (2005) 43–48.

[69] H.F. Miranda, G. Pinardi, Isobolographic analysis of the antinociceptive interactions of clonidine with nonsteroidal anti-inflammatory drugs, Pharmacol Res. 50 (2004) 273–278. doi:10.1016/j.phrs.2004.02.008.

[70] G. Pinardi, F. Sierralta, H.F. Miranda, Adrenergic mechanisms in antinociceptive effects of nonsteroidal anti-inflammatory drugs in acute thermal nociception in mice, Inflamm Res. 51 (2002) 219–222.

[71] R.B. Raffa, D.J. Stone, R.J. Tallarida, Unexpected and pronounced antinociceptive synergy between spinal acetaminophen (paracetamol) and phentolamine, Eur J Pharmacol. 412 (2001) R1–R2.

[72] H.F. Miranda, F. Sierralta, G. Pinardi, Neostigmine interactions with nonsteroidal anti-inflammatory drugs, Br J Pharmacol. 135 (2002) 1591–1597.

[73] G. Pinardi, F. Sierralta, H.F. Miranda, Atropine reverses the antinociception of nonsteroidal anti-inflammatory drugs in the tail-flick test of mice, Pharmacol Biochem Behav. 74 (2003) 603–608.

[74] R. Bjorkman, K.M. Hallman, J. Hedner, T. Hedner, M. Henning, Acetaminophen blocks spinal hyperalgesia induced by NMDA and substance P, Pain. 57 (1994) 259–264.

[75] M. Bujalska, Effect of nitric oxide synthase inhibition on antinociceptive action of different doses of acetaminophen, Pol J Pharmacol. 56 (2004) 605–610.

[76] M. Bujalska, W.S. Gumulka, Effect of cyclooxygenase and NO synthase inhibitors on antinociceptive action of acetaminophen, Pol J Pharmacol. 53 (2001) 341–350.

[77] L. Godfrey, I. Bailey, N.J. Toms, G.D. Clarke, I. Kitchen, S.M. Hourani, Paracetamol inhibits nitric oxide synthesis in murine spinal cord slices, Eur J Pharmacol. 562 (2007) 68–71.

[78] L. Godfrey, L. Yan, G.D. Clarke, C. Ledent, I. Kitchen, S.M. Hourani, Modulation of paracetamol antinociception by caffeine and by selective adenosine A2 receptor antagonists in mice, Eur J Pharmacol. 531 (2006) 80–86.

[79] J. Sawynok, A.R. Reid, Caffeine inhibits antinociception by acetaminophen in the formalin test by inhibiting spinal adenosine A_1 receptors, Eur J Pharmacol. 674 (2012) 248–254. doi:10.1016/j.ejphar.2011.10.036.

[80] D.A. Andersson, C. Gentry, L. Alenmyr, D. Killander, S.E. Lewis, A. Andersson, B. Bucher, J.L. Galzi, O. Sterner, S. Bevan, E.D. Högestätt, P.M. Zygmunt, TRPA1 mediates spinal antinociception induced by acetaminophen and the cannabinoid Δ(9)-tetrahydrocannabiorcol, Nat Commun. 2 (2011) 551. doi:10.1038/ncomms1559.

[81] H. Madenoğlu, M. Kaçmaz, R. Aksu, C. Bicer, G. Yaba, K. Yildiz, K. Doğru, A. Boyaci, Effects of naloxone and flumazenil on antinociceptive action of acetaminophen in rats, Curr Ther Res Clin Exp. 71 (2010) 111–117. doi:10.1016/j.curtheres.2010.03.001.

[82] G. Pickering, F. Moustafa, S. Desbrandes, J.M. Cardot, D. Roux, C. Dubray, Paracetamol and opioid pathways: a pilot randomized clinical trial, Fundam Clin Pharmacol. 27 (2013) 339–345. doi:10.1111/j.1472-8206.2011.01010.x.

[83] M. Bujalska, Effect of cyclooxygenase and NO synthase inhibitors administered centrally on antinociceptive action of acetaminophen (Part II), Pol J Pharmacol. 55 (2003) 1001–1011.

[84] K.S. Abelson, M. Kommalage, A.U. Hoglund, Spinal cholinergic involvement after treatment with aspirin and paracetamol in rats, Neurosci Lett. 368 (2004) 116–120.