SHORT COMMUNICATION

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# Mechanism of the Antinociceptive Effect of Paracetamol*

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## Abstract

The mechanism of action of paracetamol (acetaminophen) is still not clearly understood. Unlike morphine, for example, paracetamol has no known endogenous high-affinity binding sites. In addition, paracetamol does not appear to share with nonsteroidal anti-inflammatory drugs (NSAIDs) the capacity to inhibit peripheral cyclo-oxygenase (COX) activity. There is currently considerable evidence to support the hypothesis of a central antinociceptive effect. Although various biochemical studies point to inhibition of central COX-2 activity, the existence of a COX activity that is selectively susceptible to paracetamol (COX-3?) is an alternative hypothesis. Modulation of the serotoninergic system has also been suggested on the basis of biochemical and behavioural studies supporting an indirect serotoninergic (5-HT) effect. Paracetamol may stimulate the activity of descending 5-HT pathways that inhibit nociceptive signal transmission in the spinal cord. Support for this possibility has come from evidence that spinally administered antagonists of several 5-HT receptor subtypes abolish the antinociceptive activity of paracetamol. These hypotheses have yet to be confirmed by further studies. Until then, primary pharmacological mechanism underlying the analgesic effect of paracetamol has still to be clearly defined.

## 1. Introduction

Paracetamol or acetaminophen (*N*-acetyl-*p*-aminophenol), first synthesized in 1877 at Johns Hopkins University, was introduced into clinical practice as an analgesic as early as 1893. Subsequently discarded in favour of phenacetin, it was reintroduced in 1949. However, the mechanism underlying the antinociceptive effect of paracetamol remains largely veiled in mystery. Inhibition of peripheral cyclo-oxygenases (COX) has been suspected, although this view is currently being reappraised in the light of recent data pointing to other mechanisms, most notably central COX inhibition or indirect interaction with the serotoninergic system, or both.[1]

## 2. Conflicting Data on Peripheral COX Inhibition

Since 1971, the analgesic effect of NSAIDs, to which paracetamol was long felt to be closely connected, has been ascribed primarily to inhibition of the peripheral COX (COX-1 and COX-2).[2] However, results obtained with paracetamol have not systematically demonstrated effective inhibition of COX, which is responsible for prostaglandin

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synthesis. The *in vitro* effect of paracetamol on COX activity varies according to the study design. In cell homogenates, paracetamol has been shown to produce weak inhibition of prostaglandins or even to induce prostaglandin synthesis. Studies on intact cells have produced more positive results. Inhibition of COX activity has been achieved with micromolar concentrations similar to the plasma concentrations of paracetamol obtained in humans, although not all authors have reported the same findings. Using the human whole blood assay, paracetamol caused significant COX inhibition, again in micromolar concentrations, showing slight selectivity for COX-2.[3] However, peripheral COX-2 inhibition by paracetamol would appear to be very limited within sites of inflammation because of the high concentrations of peroxides, which are not observed *in vitro*.[4,5] In addition, the inhibitory effect of COX activity by paracetamol has been shown to increase after partial peroxide reduction by glutathione peroxidase.[4,5]

This could offer a possible explanation for the conflicting results (reduced prostaglandin concentrations in urine or in the synovial fluid of patients with rheumatoid arthritis inconsistently found in studies; *in vivo* peripheral anti-inflammatory action of paracetamol contested) obtained in studies that set out to assess indirectly the effects of paracetamol on prostaglandin metabolism.[3]

Finally, in clinical practice, paracetamol does not produce the adverse effects typically observed with NSAIDs and presumed to be induced by COX-1 inhibition (gastric toxicity, antiplatelet activity [see chapter by Graham et al.]). Paracetamol may inhibit the formation of COX-1-dependent thromboxane $A_2$, but at supratherapeutic doses.

To date, the theory of inhibition of peripheral COX activity, chiefly COX-2, by paracetamol would appear inadequate to explain the activity of this analgesic. The existence of a COX-3, a new isoform with greater sensitivity to paracetamol, has been suggested,[6] although the clinical relevance of these results and the exact sequence of the messenger RNA of this COX-3 are still under debate.[7,8]

## 3. A Documented Central Effect

### 3.1 Evidence Supporting a Central Antinociceptive Effect of Paracetamol

After systemic administration, paracetamol crosses the blood–brain barrier (approximately 40% of the plasma concentration) and is uniformly distributed in a dose-dependent manner throughout the central nervous system. The time-course of central paracetamol concentrations is comparable to that of the antinociceptive effect.[9] In addition, paracetamol also exhibits antinociceptive effects in tests that are reputed to be sensitive only to central analgesics, and intracerebroventricular or intrathecal administration of paracetamol have also been shown to provide antinociception. Finally, consistent with a central mechanism of action, paracetamol was associated with a dose-dependent reduction in the behaviour induced by intrathecal administration of various pronociceptive mediators in rats and mice.

### 3.2 Inhibition of Central COX Activity

The mechanism underlying the central antinociceptive effect of paracetamol remains unknown. Central COX inhibition was suggested when Flower and Vane[10] showed that paracetamol preferentially inhibited COX activity in the central nervous system, rather than peripherally. After treatment with paracetamol, a decline in cerebrospinal fluid prostaglandin concentrations has been reported. A dose-dependent decrease in postmortem prostaglandin $E_2$ ($PGE_2$) production has also been observed in the mouse brain, in capsaicin-infused rat spinal cord sections and in primary cultures of rat microglia after administration of lipopolysaccharide. Likewise, a dose-dependent reduction in $PGE_2$ concentrations has been demonstrated in the dorsal horn of the spinal cord of animals subjected to the formalin test, with preserved analgesic activity but no modifications in urinary $PGE_2$ excretion. However, paracetamol shows only minimal effects on $PGE_2$ and $PGF_2$ synthesis in rat brain slices or homogenates.[3]

These data would appear to point to an inhibitory effect of paracetamol on central COX activity (via a possible reduction in the oxidised active form of COX[5]), although further work is needed to elucidate the contribution of this inhibition to the antinociceptive properties of paracetamol.

### 3.3 Serotoninergic Mechanism

It was hypothesized that serotonin or 5 hydroxytryptamine (5-HT) played a part in the central analgesic mechanism of paracetamol after downregulation of this effect was observed after injury to the serotoninergic bulbospinal pathways.[11] Inhibition of 5-HT synthesis was also shown to reduce the antinociceptive activity of paracetamol.[12] Nevertheless, these studies do not show a complete loss of the effect of paracetamol. Although paracetamol has been shown to modulate serotoninergic metabolism (dose-dependent increase in tissue 5-HT concentrations in various brain structures, *in vitro* 5-HT release in rat posterior cortex sections), the mechanism underlying this interaction has still to be elucidated.[13] Inhibition by paracetamol of the hepatic enzyme indoleamine-2,3-dioxygenase has been highlighted, and this could result in an increase in brain concentrations of tryptophan, a precursor of 5-HT.[14] The finding that acute paracetamol treatment induces a decrease in the number of cortical 5-$HT_{2A}$ receptors is consistent with an increase in supraspinal 5-HT concentrations and activation of the descending inhibitory serotoninergic pathways.[12,15] In addition, the potential blocking of the 5-$HT_{1A}$ and 5-$HT_{1B}$ supraspinal somatic autoreceptors, which would appear to potentiate the effect of serotoninergic neurone activity, enhances the effect of paracetamol.[16]

Although the fine details of the causal mechanism and the degree of involvement of the bulbospinal pathways remain to be determined, the overall data are consistent with a supraspinal serotoninergic effect.

At the spinal level, a serotoninergic mechanism has also been demonstrated. In different nociceptive tests, the antinociceptive effect of paracetamol is reduced to varying degrees by intrathecal administration of 5-$HT_{1A}$, 5-$HT_{1B}$, 5-$HT_{2A}$ and 5-$HT_{2C}$ receptor antagonists.[17,18] This reversion profile is similar to that seen after intrathecal administration of 5-HT. The absence of marked affinity of paracetamol for serotoninergic receptors lends support to an indirect activation of the serotoninergic system.[19]

In conclusion, paracetamol may exert a supraspinal activity leading to a spinal effect involving 5-HT after activation of the descending serotoninergic pathways. However, evidence that bulbospinal serotoninergic pathway involvement is only partial, and the incomplete understanding of the effect of paracetamol on the serotoninergic system, clearly justify further studies in this field.

### 3.4 Other Hypotheses on Mechanisms of Action

Several studies have looked for a relationship between paracetamol and other systems involved in the regulation of nociception. Despite suggested roles for the opioid,[20] noradrenergic[21] or cholinergic[22] systems, or a possible inhibition of inducible nitric oxide synthetase,[23] other studies have yielded contradictory findings.[11,19,24]

## 4. Conclusion

Although paracetamol is nowadays the most widely prescribed analgesic in the world, its mechanism of action remains unclear. Nevertheless, recent data indicate that: (i) paracetamol is not an NSAID; (ii) peripheral COX inhibition is probably of limited magnitude within sites of inflammation; and (iii) a central site of action probably has a key role, with COX inhibition and activation of the serotoninergic system being the current hypotheses underlying this mechanism.

Numerous studies are still required, however, to provide a clearer definition of the site(s) of action of paracetamol, the nature of its effect on COX and

its modulation of serotoninergic metabolism, and of the possible relations between COX and 5-HT.

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