

# COX-3 and the mechanism of action of paracetamol/acetaminophen

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## Abstract

Paracetamol produces analgesia in the mouse writhing test through a central action which is paralleled by a reduction in brain PGE<sub>2</sub> concentrations. In contrast, diclofenac has a peripheral analgesic action in this test. Paracetamol-induced hypothermia is also accompanied by a reduction in brain PGE<sub>2</sub> concentrations in C57/Bl6 mice. This hypothermic effect of paracetamol was reduced in COX-1 but not in COX-2 gene-deleted mice. These results support the view that analgesia and hypothermia due to paracetamol are mediated by inhibition of a third COX isoenzyme (designated COX-3).

In cultured mouse macrophages, COX-2 is induced by treatment with LPS or with high concentrations of diclofenac. Diclofenac-induced COX-2 is inhibited with low concentrations of paracetamol, whereas LPS-induced COX-2 is insensitive to paracetamol inhibition. The mechanisms of induction and possibly the functions of these two COX-2 enzymes are also different.

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## 1. Introduction

In 1991, Simmons and his colleagues discovered an isoenzyme of the well known prostaglandin synthesising cyclooxygenase (COX) and named it cyclooxygenase-2 (COX-2) [1]. Herschman and his collaborators published about an inducible COX enzyme later in the same year [2]. This discovery explained the paradox of an inducible COX which had long been suspected but never identified. Eleven years later, the group of Daniel Simmons characterised and cloned a COX enzyme in dog brain which, unlike COX-1 and COX-2, was sensitive to inhibition with paracetamol (acetaminophen) [3]. This COX enzyme was a variant of COX-1 and derived from the same gene; it was designated COX-3. The importance of COX-3 is that it could explain the pharmacological actions of paracetamol and other antipyretic analgesic drugs which are weak inhibitors of COX-1 and COX-2 [4] but penetrate easily into the central nervous system [5]. Non-steroid anti-inflammatory drugs (NSAIDs), such as diclofenac or ibuprofen,

are also potent inhibitors of COX-3 expressed in cultured cells [3], but being highly polar they are unlikely to reach brain COX-3 in effective concentrations.

We have recently investigated the mechanism of the analgesic and hypothermic actions of paracetamol and connected them with inhibition of brain COX-3. We have also studied further a COX-2 variant enzyme, which is sensitive to inhibition with paracetamol. This enzyme is induced in cultured mouse macrophages by high concentrations of the NSAID, diclofenac.

## 2. Paracetamol analgesia

To measure analgesia, we used the abdominal contraction or writhing test in mice [6], since this is one of the few pain models, which is sensitive to the analgesic action of NSAIDs and paracetamol. Writhing was induced with 0.6% acetic acid or iloprost injected intraperitoneally [7] and the number of abdominal contractions occurring in the following 20 min was counted. Acetic acid-induced contractions are mediated by release of PGI<sub>2</sub> or prostacyclin [8] and those induced

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with iloprost are due to direct stimulation of IP receptors. Paracetamol inhibited dose-dependently both the acetic acid and iloprost-induced responses, whereas diclofenac inhibited only responses to acetic acid [9]. Moreover, in the acetic acid writhing test after administration of diclofenac, prostacyclin was no longer released into the peritoneal fluid, but paracetamol did not affect this release of prostacyclin. However, prostaglandin (PG) E<sub>2</sub> concentrations in the brains of mice treated with paracetamol were dose-dependently reduced whereas these concentrations only fell slightly with the highest dose of diclofenac administered. These observations indicated that paracetamol exerted a central analgesic action most likely by inhibiting COX-3, and diclofenac had a peripheral analgesic effect produced by inhibition of COX-1 [10].

Further studies have been conducted to assess the effects of selective COX-1, COX-2 and COX-3 inhibitors in the writhing test and to measure paracetamol analgesia in COX-1 and COX-2 knockout (KO) mice. Brain levels of PGE<sub>2</sub> were also measured in these investigations. Our conclusions were that paracetamol analgesia was mediated by reduction of brain PGE<sub>2</sub> synthesised by a COX enzyme derived from the COX-1 gene [unpublished observations].

### 3. Paracetamol hypothermia

In humans, paracetamol has a potent antipyretic action on fever induced with bacterial or viral infections [11]. However, bacterial lipopolysaccharide (LPS) has variable effects in rodents. It can cause a rise or a fall in body temperature or have no effect. In C57/Bl6 mice, LPS neither raised or lowered body temperature, but paracetamol demonstrated a dose-dependent hypothermic action [12]. Moreover, this hypothermic effect was paralleled by a profound decrease in PGE<sub>2</sub> concentrations in the brain. (Fig. 1). The hypothermia was reduced in COX-1 KO mice but did not differ from control in COX-2 KO animals. In COX-1 KO mice, brain levels of PGE<sub>2</sub> were only approximately 30% of those of controls and these were not reduced further by paracetamol treatment. Administration of high doses of the selective COX-1 inhibitor, SC560 or the selective COX-2 inhibitor, celecoxib did not have a hypothermic action [unpublished observations]. However, the selective COX-3 inhibitors, aminopyrine and antipyrine caused profound hypothermia in the mice [12]. With RT-PCR we were able to demonstrate mRNA for COX-3 in whole brain tissues of our mice and COX-3 enzyme protein was identified by Western blotting in different, isolated brain areas [unpublished observations].

Our conclusions from these results are that the hypothermic action of paracetamol is mediated by inhibition of COX-3 in the central nervous system most

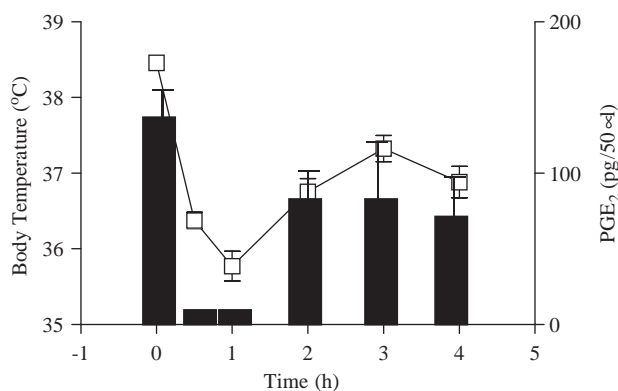


Fig. 1. The effect of 300 mg/kg paracetamol on the basal body temperature of male C57/Bl6 mice kept at an ambient temperature of 26 °C with brains removed at various times for the measurement of prostaglandin E<sub>2</sub> levels. The line graph records average basal temperature and histograms represent brain PGE<sub>2</sub> levels.

likely in the anterior hypothalamus. This would imply that a constant release of PGE<sub>2</sub> in the hypothalamic region is required to maintain the normal temperature of these animals. In humans, cats, rabbits and guinea pigs PGE<sub>2</sub> is not involved in the maintenance of a normal body temperature, but rodents have a higher thermoneutral point than these other mammals [13]. The thermoneutral temperature of mice is 32–34 °C compared to 22 °C for other mammalian species except rats. To support this thermoneutral situation at the average ambient temperature of 22 °C in which most laboratory animals are housed, a constant biosynthesis of PGE<sub>2</sub> may be required.

### 4. Is there a COX-4?

In 1995 Daniel Simmons reported [14] that high concentrations of NSAIDs applied to cultured chick fibroblasts induced COX-2 and apoptosis in these cells. A follow up report in 1999 described the induction of a COX-2-like enzyme in cultured J774.2 mouse macrophages after incubation with high concentrations of diclofenac for 48 h [15]. This diclofenac-induced COX-2 was more sensitive to inhibition by paracetamol than LPS-induced COX-2. On further investigation, Simmons discovered that LPS-induced COX-2 was a membrane-bound enzyme and COX-2 induced with diclofenac existed in the cytoplasm of cells. At this time it was also found that in experimental carrageenan pleurisy in the rat a late-induced COX-2 appeared during the resolution phase of the inflammation [16]. 15-deoxy-PGJ<sub>2</sub> synthesised by this enzyme would then be involved in resolving the inflammation in this model and administration of paracetamol prevented resolution (unpublished observation). Preliminary experiments have shown that while having no effect on the onset of

inflammation, paracetamol may inhibit the late-induced COX-2.

We have continued an investigation comparing the different properties of LPS and diclofenac-induced COX-2 enzymes. We have found that LPS induces COX-2 in J774.2 macrophages by activation of NF $\kappa$ B, whereas diclofenac induction of COX-2 is mediated by activation of the PPAR $\gamma$  nuclear receptor. Also, macrophages stimulated with LPS release PGE<sub>2</sub> as well as pro-inflammatory cytokines, while after stimulation with diclofenac PGE<sub>2</sub> and anti-inflammatory cytokines are formed by J774.2 cells [unpublished observations].

## 5. Conclusions

Paracetamol very likely produces analgesia and hypothermia in mice by inhibiting COX-3 in the central nervous system and lowering PGE<sub>2</sub> levels. Inhibition of an inducible COX, perhaps COX-4, may also mediate some of the actions of paracetamol but this putative enzyme has so far only been characterised in isolated cells.

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