ANALGESICS

New clues in the acetaminophen mystery

Although acetaminophen (paracetamol) has been used clinically for more than a century, its mode of action is still not clear. Writing in the *Journal of Biological Chemistry*, Zygmunt and colleagues have now provided evidence for a new and unexpected mechanism through which acetaminophen could exert its analgesic effects.

Acetaminophen differs significantly from aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs), with which it is often grouped because of their shared analgesic and antipyretic effects, as it is only a weak anti-inflammatory agent and has a low incidence of gastric side effects. The effects of NSAIDs are thought to depend on their ability to inhibit two forms of cyclooxygenase, COX1 and COX2, and the consequent inhibition of the synthesis of prostaglandins. However, despite much research, definitive proof that the analgesic and antipyretic effects of acetaminophen are dependent on COX is still lacking. Indeed, inhibition of a third form of COX, COX3, is one of the more recent proposals that has been put forward to explain the unusual effects of acetaminophen, but further analysis has suggested that this interaction is unlikely to be clinically relevant.

There have also been indications that the analgesic effects of acetaminophen are mediated by molecular targets distinct from COX, and it was this path of investigation that was followed by Zygmunt and colleagues. The stimulus for their studies was the striking relationship between the structures of acetaminophen and the *N*-acyl phenolamine AM404, which is both a potent activator of the ion channel $TRPV_1$ and has effects on cannabinoid CB_1 receptors. Both $TRPV_1$ and CB_1 receptors are involved in pain and thermoregulatory pathways and are viewed as promising targets for the treatment of pain and inflammation.

The structural relationship between AM404 and acetaminophen suggested that following deacetylation to its metabolite *p*-aminophenol, acetaminophen could be conjugated with arachidonic acid to give AM404. The authors provided several lines of evidence to support this idea, including demonstrating that deuterium-labelled AM404 and *p*-aminophenol were dose-dependently formed in rat brain after the administration of deuterium-labelled acetaminophen at doses that produce analgesia in rodents. AM404 could also be detected in the spinal cord of rats given acetaminophen and *p*-aminophenol.

Furthermore, the authors provide evidence for the pathway by which AM404 is formed, by showing that fatty acid amide hydrolase (FAAH), which is known to hydrolyse endogenous compounds related to AM404, can act in the reverse direction, and synthesize AM404 from *p*-aminophenol and arachidonic acid *in vitro*. In addition, no formation of AM404 was observed *in vitro* or *in vivo* in brain tissue from mice that lacked FAAH.

Finally, the authors also showed that AM404 inhibits purified COX1 and COX2 and prostaglandin formation in lipopolysaccharide-stimulated macrophages. In summary, the identification of AM404 as a novel metabolite of acetaminophen in the nervous system that affects several important targets involved in pain and thermoregulatory pathways provides a new hypothesis for explaining the acetaminophen mystery. The confirmation of the relevance of this hypothesis to the pharmacological effects of acetaminophen in humans will surely be eagerly anticipated.

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References and links

ORIGINAL RESEARCH PAPER Hogestatt, E. *et al.* 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**, 31405–31412 (2005)