Acetaminophen Inhibits Spinal Prostaglandin E₂ Release after Peripheral Noxious Stimulation

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Background: Prostaglandins play a pivotal role in spinal nociceptive processing. At therapeutic concentrations, acetaminophen is not a cyclooxygenase inhibitor. Thus, it is antinociceptive without having antiinflammatory or gastrointestinal toxic effects. This study evaluated the role of spinal prostaglandin E₂ (PGE₂) in antinociception produced by intraperitoneally administered acetaminophen.

Methods: The PGE₂ concentrations in the dorsal horn of the spinal cord were measured after formalin was injected into the hind paw of rats. The effect of antinociceptive doses of acetaminophen (100, 200, and 300 mg/kg given intraperitoneally) on PGE₂ levels and flinching behavior was monitored. Spinal PGE₂ and acetaminophen concentrations were obtained by microdialysis using a probe that was implanted transversely through the dorsal horn of the spinal cord at L₄. Furthermore, the effects of acetaminophen on urinary prostaglandin excretion were determined.

Results: Intraperitoneal administration of acetaminophen resulted in a significant decrease in spinal PGE₂ release that was associated with a significant reduction in the flinching behavior in the formalin test. Acetaminophen was distributed rapidly into the spinal cord with maximum dialysate concentrations 45–60 min after intraperitoneal administration. Urinary excretion of prostanoids (PGE₂, PGF₂α, and 6-keto-PGF₁α) was not significantly altered after acetaminophen administration.

Conclusions: The data confirm the importance of PGE₂ in spinal nociceptive processing. The results suggest that antinociception after acetaminophen administration is mediated, at least in part, by inhibition of spinal PGE₂ release. The mechanism, however, remains unknown. The finding that urinary excretion of prostaglandins was not affected might explain why acetaminophen is antinociceptive but does not compromise renal safety. (Key words: Eicosanoids; nociception; paracetamol; spinal nociceptive processing.)

Despite its widespread use for pain in various clinical conditions, the mechanism of the analgesic action of acetaminophen is still poorly defined. Although the drug is often classified as a nonsteroidal antiinflammatory drug, its weak antiinflammatory activity and weak inhibition of peripheral prostaglandin biosynthesis differ clearly from those of other nonsteroidal antiinflammatory drugs.¹⁻⁴

There is now increasing evidence of a central mechanism of action of acetaminophen.⁵⁻⁷ Its pKa of 9.5, and thus its largely unionized form in the physiologic pH range, its low plasma-protein binding,¹ and the ability to cross the human and rat blood–brain barrier² are compatible with a central effect shown in several experimental and clinical studies.⁹⁻¹⁴ Although acetaminophen does not inhibit cyclooxygenases in vitro at therapeutic concentrations, initial studies suggested that it may be particularly active in inhibiting prostaglandin synthesis in the central nervous system.¹⁵⁻¹⁷

Recent research showed that acyclooxygenase isoenzymes are present in the central nervous system and that both enzymes are active in the spinal cord.¹⁸ Furthermore, prostaglandin E₂ (PGE₂) reportedly is released from the spinal cord of rats after peripheral noxious stimulation in the formalin assay.¹⁹⁻²⁰

The formalin test allows the response to moderate continuous stimulation to tissue injury to be assessed and is considered to provide a valid model of clinical pain.²¹

The purpose of the current study was to determine whether the antinociceptive effect of acetaminophen in the formalin assay can be attributed, at least in part, to changes in spinal PGE₂ release. Therefore, microdialys-
sis\textsuperscript{22} was applied to assess interstitial concentrations of PGE\textsubscript{2} in the dorsal horn of the lumbar spinal cord of conscious, freely moving rats.\textsuperscript{20,22} To evaluate the effect of acetaminophen on prostaglandin synthesis in another organ, we studied the urinary excretion of PGE\textsubscript{2}, PGF\textsubscript{2\alpha}, and 6-keto-PGF\textsubscript{1\alpha}.

Materials and Methods

Chemicals
Acetaminophen was purchased from Sigma Chemie (Deisenhofen, Germany). All other chemicals and solvents were of HPLC or reagent grade and were purchased from Sigma Chemie.

Animals
Male Sprague-Dawley rats (weight, 280–320 g; Charles River, Sulzfeld, Germany) were used in all experiments. They were housed in groups of six and were maintained on a 12-h light-dark cycle with free access to food and water. Experiments were approved by the local ethics committee and conformed to the guidelines set out by the International Association for the Study of Pain.

Implantation of the Spinal Microdialysis Probes
Animals were administered 100 mg/kg ketamine (Pharmacia & Upjohn GmbH, Erlangen, Germany) and 5 mg/kg xylazine intraperitoneally (Bayer AG, Leverkusen, Germany) to induce anesthesia and for intraoperative analgesia. During surgery, they were deeply anesthetized by a constant flow of isoflurane (1–1.5 vol%; Abbott GmbH, Wiesbaden, Germany). A controlled heating pad (CMA/Microdialysis, Stockholm, Sweden) kept the animals’ core temperature at 37°C. An incision was made above the vertebral column, and muscle tissue was cleared away from vertebrae T13 and L1. The animals were secured in stereotaxic frames (David Kopf Instruments, Tujunga, CA). Small holes were drilled (using a diamond drill) through the lateral surface of vertebra T13 at the level of the dorsal horn. A dialysis tube constructed from a Cuprophan hollow fiber (diameter, 200 μm; molecular weight cutoff, approximately 40 kd; Hospital, Nürnberg, Germany) was placed through the holes, passing transversely through the dorsal horn of the spinal cord, as described previously.\textsuperscript{20,25} The dialysis membrane was covered thoroughly with epoxy glue, except for the part located in the spinal cord tissue. The ends of the dialysis tube were connected to polyethylene tubes, which were passed subcutaneously to the neck, where they were fastened. At the end of surgery, 50 mg/kg ketamine and 2.5 mg/kg xylazine were injected intraperitoneally for postoperative analgesia. After a 24-h recovery period in individual cages, the animals were evaluated for any signs of limb paralysis or impaired movement. Rats that showed any neurologic deficit after surgery were killed immediately. All rats were used only once for dialysis experiments.

Microdialysis in the Spinal Cord
After a 24-h recovery period, the dialysis system was connected to a microdialysis pump (CMA 100) and perfused with artificial cerebrospinal fluid (ACSF: 141.7 mM Na\textsuperscript{+}, 2.6 mM K\textsuperscript{+}, 0.9 mM Mg\textsuperscript{2+}, 1.3 mM Ca\textsuperscript{2+}, 122.7 mM Cl\textsuperscript{−}, 21 mM HCO\textsubscript{3}−, 2.5 mM HPO\textsubscript{4}\textsuperscript{2−}, 3.5 mM dextrose, bubbled with 5% carbon dioxide in 95% oxygen to adjust the pH to 7.2, preheated to 37°C) at a flow rate of 5 μl/min. Animals were placed in a freely moving chamber (CMA Freely Moving System, CMA/Microdialysis) and samples were collected at 15-min intervals and stored at −70°C for subsequent analysis of immunoreactive PGE\textsubscript{2} (irPGE\textsubscript{2}) and acetaminophen. After a washout period of 60 min to allow for tissue recovery, baseline samples were collected during a 60-min period. Acetaminophen (100 mg/kg, n = 6; 200 mg/kg, n = 4; and 300 mg/kg, n = 5) was injected intraperitoneally in a randomized and blinded manner. Thirty minutes after drug administration 50 μl 5% formalin was injected subcutaneously into the dorsal surface of the right hind paw. Control groups received either 50 μl formalin, 5% (n = 6) or 50 μl saline (n = 3) without intraperitoneal drug administration. Dialysate samples were collected for at least 7.5 h. Sampling times were corrected for the dead space volume of the outlet tubing.

After formalin injection, flinches of the injected paw were counted in 1-min intervals for 60 min.\textsuperscript{21} Counts were done by the same observer in all rats in a blinded manner.

After each experiment, the animals were killed with an overdose of pentobarbital, and patency (injection of methylene blue solution) and catheter placement (in the dorsal horn) were checked by microscopic examination.

The following exclusion criteria were predefined. Each animal with neurologic or behavioral deficits was killed and replaced. Animals with a variable baseline for irPGE\textsubscript{2} (i.e., at least one concentration differing more than two standard deviations from the mean baseline value) were excluded from the statistical analysis and not replaced because of the randomized design of the study.

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Characterization of Dialysis Efficiency

To estimate absolute concentrations of acetaminophen in spinal cord tissue, the recovery of the dialysis membrane was determined by two different calibration procedures.

In Vitro. The relative recovery of the dialysis membrane was determined in ACSF spiked with two concentrations of acetaminophen (0.5 and 10 µg/ml). The solutions were maintained at 37°C and stirred constantly. The flow rate was 5 µl/min; the sampling interval was 15 min, and the total sampling time was 3 h for each concentration. The concentrations of acetaminophen in the dialysate (C_dialysate) were measured, and the recovery was calculated as rec(%) = 100 · (C_dialysate/C_spiked_ACSF).

In Vivo. In three rats, the in vivo recovery of the spinal dialysis membrane was assessed by retrodialysis.24 25 The perfusate (ACSF) was spiked with five concentrations of acetaminophen (0.1, 1, 10, 50, and 300 µg/ml), and the delivery was monitored for at least 1 h (flow rate 5 µl/min). The delivery was calculated as del(%) = 100 - [100 · (C_dialysate/C_perfusate)].

Urine Sampling

To assess the influence of acetaminophen on prostaglandin synthesis in another organ in vivo, we collected spontaneously micturated urine 2.5 h before and 2.5 h after intraperitoneally injected acetaminophen (two groups of rats: 200 mg/kg, n = 5; 300 mg/kg, n = 6). Immunoreactive prostaglandin E₂, 6-keto-prostaglandin F₁α (ir6-keto-PGF₁α), and PGF₂α concentrations were determined in the sample before and after acetaminophen was administered. In these experiments, no formalin or saline was injected.

Determination of Acetaminophen Concentrations

Acetaminophen concentrations in the dialysate were determined by HPLC,24 as described previously. The limit of quantification of the assay was 50 ng/ml. The calibration curve showed a linear response (r < 0.99) for the concentration range (50–40,000 ng/ml) tested. The coefficient of variation for the calibration range was less than 4%.

Measurement of Immunoreactive Prostaglandin E₂

Immunoreactive 6-keto-prostaglandin F₁α, and Immunoreactive Prostaglandin F₁α

Immunoreactive PGE₂ concentrations in urine and dialysate and ir6-keto-PGF₁α and irPGF₂α concentrations in urine were determined using commercially available enzyme immunoassay kits (Cayman Chemicals, Ann Arbor, MI). The limits of quantification were 20 pg/ml, 15 pg/ml, and 14 pg/ml, respectively. The coefficients of variation for the calibration range of 20–1,000 pg/ml (irPGE₂), 15–500 pg/ml (ir 6-keto-PGF₁α), and 15–1,000 pg/ml (irPGF₂α) were less than 14%, 12%, and 15%, respectively.

Data Analysis

Maximum acetaminophen concentrations in spinal dialysate and the time to reach these concentrations were taken directly from the data.

The mean irPGE₂ concentrations in spinal dialysate before administration of acetaminophen were defined as basal levels. For comparison of drug effects on the irPGE₂ dialysate concentrations, the areas under the irPGE₂ concentration time curves (AUC) for the first hour after formalin injection and the following 5 h, respectively (AUC₀–₁h and AUC₁₂₅–₆h), were calculated according to the linear trapezoidal rule.

The time course of the flinching behavior was classified as phases 1, 2a, and 2b, which is an accepted method to differentiate between immediate and prolonged nociceptive behavior in the formalin test.9 21 Flinching data for each rat were summarized in 5-min intervals as mean flinches/min and then plotted as group mean ± SD versus time.

The amount of prostaglandins excreted in urine was obtained by multiplying the respective urine concentration by the urine volume for 2.5 h.

Statistical Analyses

Group data are presented as the mean ± SD. Statistical analysis was performed using SPSS 8.0 for Windows (SPSS, Chicago, IL). To assess differences between treatments (formalin, formalin + 100 mg/kg acetaminophen, formalin + 200 mg/kg acetaminophen, formalin + 300 mg/kg acetaminophen, saline) the areas under the irPGE₂ concentration time curves for the first hour after formalin injection (AUC₀–₁h) were evaluated by analysis of variance and the Bonferroni t tests as post hoc tests. Analysis of variance was repeated for the AUCs from 1.25 to 6 h after formalin injection (AUC₁₂₅–₆h).

Total flinches during phases 1, 2a, and 2b were evaluated by analysis of variance. Again, analysis of variance was followed by the Bonferroni post hoc test.

To assess (1) the effects of acetaminophen on the excretion of irPGE₂, irPGF₂α, and 6-keto-PGF₁α in urine and (2) differences between both acetaminophen doses, prostaglandin amounts in urine were evaluated by analysis of variance. The within-subject factor was "drug
administration" (before and after), and the between-subject factor was “dose” (200 and 300 mg/kg acetaminophen). The alpha level was set to 0.05.

**Results**

*Animals Not Included in the Statistical Analyses*

Two animals were excluded and replaced because of minor neurologic deficits. Three animals with variable baseline irPGE$_2$ levels were excluded but not replaced. *Post mortem* examinations revealed damaged microdialysis fibers in two of these animals. No microdialysis fiber was located outside the target area as verified by microscopic examination of the spinal cord after the dialysis experiment.

**Efficiency of Dialysis**

The *in vitro* recovery obtained by using ACSF spiked with 500 ng/mL and 10 µg/mL acetaminophen were 17.8% ± 0.5% and 16.8% ± 0.1%, respectively. The *in vivo* delivery was linear for the concentration range tested (0.1, 1, 10, 50, and 300 µg/mL), with a mean delivery of 17.5% ± 1.5%. Thus, tissue concentrations of acetaminophen are estimated to be 5.7 times those of spinal dialysate concentrations.

**Spinal Prostaglandin E$_2$ Release after Formalin Injection and Flinching Behavior**

After formalin injection, there was a remarkable increase of irPGE$_2$ in the dialysate samples compared with saline injection. Immunoreactive PGE$_2$ levels rapidly returned to baseline values approximately 1 h after formalin injection (fig. 1A). Analysis of variance with subsequent Bonferroni *post hoc* tests showed a significant difference for the first hour AUC$_{0-1h}$ between formalin and saline ($P = 0.015$; 95% CI for differences, 22.42-281.09). Formalin injection produced a characteristic biphasic flinching behavior of the injected paw, with an initial acute phase (0-10 min, phase 1) followed by a second phase (phase 2) with a maximum between 20 and 40 min after formalin injection (fig. 1B).

**Flinching Behavior and Spinal Prostaglandin E$_2$ Release after Acetaminophen Administration**

Administration of acetaminophen 30 min before formalin injection prevented the formalin-evoked increase of PGE$_2$ dialysate concentrations. Interestingly, both higher acetaminophen doses (200 and 300 mg/kg) led to a dose-dependent reduction of PGE$_2$ concentrations even below basal levels. After 200 mg/kg acetaminophen, irPGE$_2$ levels returned to baseline values approximately 5 h after formalin injection. After 300 mg/kg they remained below baseline values until the end of the sampling period. The time course of spinally released irPGE$_2$ after intraperitoneally administered acetaminophen (100, 200, 300 mg/kg) is shown in figures 2A, 3A, and 4A, respectively. Analysis of variance with subsequent Bonferroni *t* tests revealed a significant difference for the first hour AUC$_{0-1h}$ between formalin injection alone and both higher acetaminophen doses (200 mg/kg: $P = 0.002$; 95% CI for differences, 53.75-293.23, 300 mg/kg: $P = 0.001$; 95% CI for differences, 75.11-302.31). The first hour AUC$_{0-1h}$ after 100 mg/kg acetaminophen was not significant compared with formalin injection alone ($P = 0.051$; 95% CI for differences, -0.040 to 218.22).
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100 mg kg\textsuperscript{-1} Acetaminophen

![Graph A: Concentration versus time curves for irPGE\textsubscript{2} and acetaminophen in spinal microdialysate before and after administration of 100 mg/kg acetaminophen (n = 6). The arrow indicates the time of formalin injection (0.5 h after acetaminophen administration).](image)

200 mg kg\textsuperscript{-1} Acetaminophen

![Graph B: Concentration versus time curves for irPGE\textsubscript{2} and acetaminophen in spinal microdialysate before and after administration of 200 mg/kg acetaminophen (n = 4). The arrow indicates the time of formalin injection (0.5 h after acetaminophen administration).](image)

![Graph C: Behavior data and immunoreactive PGE\textsubscript{2} concentrations in spinal microdialysate before and after formalin injection.](image)

The reduction of PGE\textsubscript{2} levels was associated with an inhibition of the typical flinching behavior in phases 1 and 2a, which is shown in figures 2B, 3B, and 4B. In phase 2a, sums of flinches were significantly reduced for all acetaminophen doses (100 mg/kg: \( P = 0.011 \); 200 mg/kg: \( P = 0.001 \); 300 mg/kg: \( P < 0.001 \)). In phase 1, only 200 and 300 mg/kg acetaminophen led to a significant reduction of the flinching behavior (200 mg/kg: \( P = 0.001 \); 300 mg/kg: \( P < 0.001 \)).

After administration of 300 mg/kg acetaminophen, the irPGE\textsubscript{2} AUC from 1.25 to 6 h (AUC\textsubscript{1.25-6}) was reduced compared with the other treatment groups (table 1). The difference between 100 and 300 mg/kg acetaminophen was significant (\( P = 0.034 \), 95% CI for differences, 21.80–771.05).

**Urinary Prostaglandin Excretion after Acetaminophen Administration**

Urinary excretion of major renal prostanoids was not significantly influenced by acetaminophen at doses between 200 and 300 mg/kg (irPGE\textsubscript{2}: \( P = 0.266 \); ir6-keto-PGF\textsubscript{1α}: \( P = 0.063 \); irPGF\textsubscript{2α}: \( P = 0.472 \); fig. 5). In addition, there was no significant difference between the two doses of acetaminophen (irPGE\textsubscript{2}: \( P = 0.977 \); ir6-keto-PGF\textsubscript{1α}: \( P = 0.521 \); irPGF\textsubscript{2α}: \( P = 0.426 \)).

Fig. 3. (A) The concentration versus time curves (mean ± SD) of immunoreactive prostaglandin E\textsubscript{2} (PGE\textsubscript{2} ; ○) and acetaminophen (●) in spinal microdialysate before (1-h baseline collection) and after intraperitoneal administration of 200 mg/kg acetaminophen (n = 4). The arrow indicates the time of formalin injection (0.5 h after acetaminophen administration). (B) Behavioral data (mean flinches/min ± SD at 5-min intervals, ◦) and immunoreactive PGE\textsubscript{2} concentrations (mean ± SD, ○) 1 h after formalin injection.

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Discussion

Implantation of a dialysis fiber directly into spinal cord tissue is a well-established technique in various animal species, such as cats, rats, and sheep. It offers the advantage to localize the origin of mediators more precisely. Spinal release of PGE$_2$ in rats was previously measured using an in vitro perfusion technique, spinal microdialysis, or intrathecal loop-dialysis. In the current study, we used a dialysis probe inserted transversally through the dorsal horn of the lumbar spinal cord (L4) to assess extracellular PGE$_2$ concentrations at the region of afferent C-fiber input. This in vivo method allows for simultaneous monitoring of spinal PGE$_2$ and acetaminophen concentrations in the spinal cord after formalin injection into the hind paw of rats.

Acetaminophen is a widely used systemic analgesic. The three primary pharmaceutical manufacturers estimate that, in 1997, approximately 29,000 tons of acetaminophen were consumed in the United States. One of its major advantages is its low frequency of side effects compared with other nonopioid analgesics. A serious side effect, however, is its ability to produce severe dose-related hepatotoxicity, which can be life-threatening. To avoid hepatotoxicity, doses must not exceed manufacturer recommendations.

The mechanism of action of acetaminophen is still poorly defined. Although acetaminophen has been shown to be an effective drug in antagonizing prostaglandin-mediated edema, it does not inhibit cyclooxygenases in vitro at therapeutic concentrations. Research has suggested that it may selectively act as a more potent inhibitor of prostaglandin synthesis in the central nervous system rather than in the periphery. Systemically administered acetaminophen has been shown to inhibit ischemia-induced PGE$_2$ formation in the rat brain. The degree of inhibition of brain cyclooxygenase correlated well with antinociceptive potency in the acetylcholine-induced constriction test. Similarly, some other studies indicated an inhibition of prostaglandin synthesis in the central nervous system using microsomal preparations or brain homogenates. In contrast, Lanz et al. could not find tissue-specific differences in the cyclooxygenase sensitivities of acetaminophen using astrocyte and peritoneal macrophage preparations.

Peripheral nociceptive stimulation by subcutaneous formalin injection into the hind paw evoked a concurrent increase of extracellular PGE$_2$ concentrations. In contrast, injection of saline into the hind paw did not change spinal extracellular PGE$_2$ levels. These findings confirm the substantial role of spinally released PGE$_2$ in spinal nociceptive processing, as suggested previously.

In the current study, antinociceptive doses of acetaminophen significantly inhibited the formalin-evoked PGE$_2$ release in the dorsal horn of the rat spinal cord. As expected, the number of flinches in the formalin test was clearly reduced. Both higher doses (i.e., 200 and 300 mg/kg) of acetaminophen did not inhibit urinary excretion of PGE$_2$, PGF$_{1a}$, or PGF$_{2a}$, significantly, indicating that peripheral prostaglandin synthesis was not affected. In contrast to acetaminophen, nonsteroidal antiinflammatory drugs such as acetylsalicylic acid, ketoprofen, or
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Table 1. Comparison of PGE₂ Concentrations in Spinal Dialysate of the First Dialysate Sample after Formalin or Saline Injection and the irPGE₂ AUCs during the First Hour (AUC₀₋₁₅) and from 1.25 to 6 Hours (AUC₁.₂₅₋₆) after Formalin Injection

<table>
<thead>
<tr>
<th>Condition</th>
<th>irPGE₂ 15 min (pg/ml)</th>
<th>AUC₀₋₁₅ (pg · ml⁻¹ · h⁻¹)</th>
<th>AUC₁.₂₅₋₆ (pg · ml⁻¹ · h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin = control (n = 6)</td>
<td>304.6 ± 137.3</td>
<td>243.8 ± 77.2</td>
<td>437.3 ± 43.0</td>
</tr>
<tr>
<td>Saline (n = 3)</td>
<td>94.2 ± 29.5</td>
<td>92.1 ± 14.7</td>
<td>415.5 ± 94.9</td>
</tr>
<tr>
<td>Formalin + acetaminophen 100 mg/kg ip (n = 6)</td>
<td>165.7 ± 99.1</td>
<td>134.9 ± 68.8</td>
<td>552.9 ± 266.4</td>
</tr>
<tr>
<td>Formalin + acetaminophen 200 mg/kg ip (n = 4)</td>
<td>89.4 ± 48.1</td>
<td>70.3 ± 30.9</td>
<td>345.1 ± 253.2</td>
</tr>
<tr>
<td>Formalin + acetaminophen 300 mg/kg ip (n = 5)</td>
<td>67.1 ± 27.7</td>
<td>55.1 ± 23.1</td>
<td>156.5 ± 33.5</td>
</tr>
</tbody>
</table>

Ibuprofen have been shown to inhibit renal prostaglandin synthesis at therapeutic doses, which is thought to be responsible for the renal toxicity observed with these drugs.³⁵⁻³⁸

Thus, our observations correspond with the suggestion that the antinociceptive effect of acetaminophen is due, at least in part, to an inhibition of prostaglandin release in the spinal cord. However, the observed reduction of spinal PGE₂ concentrations could be the result of unspecific effects such as an increased tissue clearance or metabolism of spinal PGE₂ by acetaminophen.

In addition to prostaglandin release, peripheral nociceptive stimulation has been shown to induce a release of glutamate, substance P, and other mediators that lead to an increase of intracellular calcium concentrations and phospholipase activation in the projection neurons.³⁹⁻⁴² Bjorkman et al.¹⁴ found an antagonistic effect of acetaminophen (200 mg/kg given intraperitoneally) in a model of hyperalgesia induced by intrathecal administration of N-methyl-D-aspartate and substance P. In the same study, the antinociceptive effect of acetaminophen was reversed by administration of L-arginine, the natural substrate for nitric oxide synthase. Whether acetaminophen acts centrally via a cross-talk between spinal cyclooxygenases and the nitric oxide synthase pathway or other mediators should be investigated further. Evidence, however, exists that serotonergic mechanisms are involved in acetaminophen-mediated antinociception. Pelissier et al.¹⁰ reported that the antinociceptive action of acetaminophen was inhibited by the intrathecal administration of tropisetron, a 5-hydroxytryptamine₃ receptor antagonist, whereas Pini et al.⁹ showed that antinociception mediated by acetaminophen was accompanied by a significant, naloxone-reversible increase in brain serotonin concentrations. Whether the observed inhibition of the formalin-evoked spinal PGE₂ release by acetaminophen can be attributed to an antagonism of excitatory transmitters or a potentiation of central serotonergic mechanisms or both is still not known. Further research is necessary to clearly define the molecular mechanisms of inhibition of spinal PGE₂ release and to determine how this knowledge would then fit into current knowledge of spinal cord pharmacology of pain research.¹¹
In conclusion, our data support the view that acetaminophen exerts some of its antinociceptive activity by inhibiting spinal prostaglandin release. Our results give further evidence of a central mechanism of the antinociceptive action of acetaminophen. At the concentrations used, acetaminophen inhibited the release of prostaglandins in the spinal cord but not in the kidney. Thus, these data confirm that major renal safety problems associated with prostaglandin synthesis inhibition are unlikely to occur with acetaminophen at antinociceptive doses.

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