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Acetaminophen: A Central Analgesic Drug That Involves a Spinal Tropisetron-Sensitive, Non–5-HT_3 Receptor-Mediated Effect

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ABSTRACT

The reversal of the antinociceptive effect of systemically administered acetaminophen (paracetamol) by intrathecal administration of the potent 5-HT_3 receptor antagonist tropisetron has been reported in rats subjected to the paw pressure test, suggesting that acetaminophen action is mediated through spinal 5-HT_3 receptors. However, more recent data, showing differences between the pharmacological profiles of various 5-HT_3 receptor antagonists, led us to reconsider the involvement of spinal 5-HT_3 receptors. To address this question, two different approaches were used: 1) electrophysiological recordings to assess whether acetaminophen directly modulates 5-HT_3 receptor activity and 2) pharmacological investigations with various 5-HT_3 receptor antagonists and spinal 5-HT_3 receptors antisense oligodeoxynucleotides (AODNs) to determine how those treatments might affect the antinociceptive action of acetaminophen. Electrophysiological studies demonstrated that acetaminophen had no direct agonist or antagonist effects on 5-HT_{3A} receptors. Unlike tropisetron, other 5-HT_3 receptor antagonists, such as ondansetron and granisetron, injected intrathecally were unable to reverse the antinociceptive effect of acetaminophen. Moreover, pretreatment with AODNs did not reverse the acetaminophen-induced antinociceptive effect, although it suppressed the antinociceptive effect of *m*-chlorophenylbiguanide, a specific agonist of 5-HT_3 receptors, and significantly reduced (30%) the expression of these receptors in the dorsal horn of the spinal cord. These results suggest that acetaminophen-induced antinociceptive action involves a spinal tropisetron-sensitive receptor that is not the 5-HT_3 receptor and that remains to be identified.

The mechanism of the analgesic action of acetaminophen is not yet clear. Some authors have considered a peripheral local action, but this possibility is not supported by other studies (for review, see Bonnefont et al., 2003). Physicochemical, pharmacokinetic, and pharmacodynamic data are more in favor of a central effect of this molecule: acetaminophen 1) crosses the blood-brain barrier (Bannwarth et al., 1992; Courade et al., 2001a); 2) induces an antinociceptive effect after central administration (Gelgor et al., 1992; Alloui et al., 1996; Pélissier et al., 1996); and 3) inhibits responses in noninflammatory pain models after systemic administration (Hunskaar et al., 1985; Pélissier et al., 1996).

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Various mechanisms have been evoked to explain the central action of acetaminophen, such as inhibition of central cyclooxygenases (Chandrasekharan et al., 2002) and/or inhibition of NO synthase (Ryu et al., 2000), but their contribution to its analgesic effect is a matter of debate (Mitchell et al., 1993; Amin et al., 1995; Farivar et al., 1996; Warner et al., 1999). Another evoked mechanism implicates the serotonergic system. Indeed, lesions of serotonergic pathways (Tjolsen et al., 1991) as well as inhibition of serotonin (5-HT, 5-hydroxytryptamine) synthesis by *p*-chlorophenylalanine (Pini et al., 1996) markedly reduced the antinociceptive effect of acetaminophen. On the other hand, acetaminophen treatment was shown to increase 5-HT levels in various central nervous system structures (Pini et al., 1996; Courade et al., 2001b) and reduce the density of cortical 5-HT_2 receptors (Pini et al., 1996; Srikiatkhachorn et al., 1999). Even though

ABBREVIATIONS: AODN, antisense oligodeoxynucleotides; mCPBG, *m*-chlorophenylbiguanide; ODN, oligodeoxynucleotides; AUC, areas under the time course curves.

both pro- and antinociceptive effects of serotonin can exist (for review, Millan, 2002), these data suggest that a serotonergic mechanism might be involved in the antinociceptive action of paracetamol.

In 1996, Pelissier et al. reported that the antinociceptive effect of systemically administered acetaminophen could be reversed by tropisetron, a 5-HT_3 receptor antagonist. They suggested that the 5-HT_3 receptor, which has been reported to have both pro and antinociceptive influences in the spinal cord (Bardin et al., 1997; Zeitz et al., 2002), could be involved in the antinociception mediated by acetaminophen. However, other data show that tropisetron is not a selective 5-HT_3 receptor antagonist (Arranz et al., 1998; Maksay et al., 1999; Macor et al., 2001) and led us to reconsider the actual implication of 5-HT_3 receptors in the antinociceptive action of acetaminophen. For this purpose, two series of experiments were performed: 1) in vitro electrophysiological recordings to assess whether acetaminophen directly activates 5-HT_3 receptors and 2) in vivo pharmacological investigations aimed at assessing whether various 5-HT_3 receptor antagonists and antisense oligodeoxynucleotides (AODNs) that down-regulate 5-HT_3 receptor expression interfere with the antinociceptive action of acetaminophen.

Materials and Methods

In Vitro Studies

Transient Expression of 5-HT_{3A} Receptor. The cDNA encoding the mouse 5-HT_{3A} receptor (Maricq et al., 1991) was inserted in the vertebrate expression vector pcDNA3 (Invitrogen). For transient expression in stage V and VI *Xenopus laevis* oocytes, nuclear injection was performed as described previously (Bourinet et al., 1994) with 10 nl of the 5-HT_{3A} encoding plasmid suspension. Oocytes were then incubated at 18°C for 2 to 4 days in ND96 medium on a rotating platform.

Electrophysiology. Macroscopic oocyte currents were recorded using two voltage-clamp electrodes with a GeneClamp500 amplifier (Axon Instruments, Union City, CA) in ND96 solution containing 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 2.5 mM sodium pyruvate, and 5 mM HEPES, pH adjusted to 7.45 with NaOH (Bourinet et al., 1994). Oocytes were placed in a small recording chamber continuously perfused by a gravity-driven perfusion system controlled by solenoid valves. The system allows a fast application of solutions. pClamp7 software (Axon Instruments) was used for data acquisition, and analysis was performed with pClamp8, Excel (Microsoft Corp. Redmond, WA), and Prism (GraphPad Software, San Diego, CA) software.

Three experiments were performed: a first based on the application of 10 μM 5-HT at different holding potentials to reveal the functionality of 5-HT_{3A} receptors; a second with 5-HT and the 5-HT_3 receptor antagonist, granisetron to confirm the involvement of these receptors; and a third to comparatively assess the effect of 5-HT, *m*-chlorophenylbiguanide (mCPBG), and acetaminophen on the 5-HT_3 receptors. To be exhaustive, a last experiment was performed to determine whether acetaminophen could act as a 5-HT_3 receptor antagonist. $n = 5$ to 32 according to the experiments.

In Vivo Studies

Animals. For all experiments, male Sprague-Dawley rats (180–200 g) were used (Charles River Laboratories, St Aubin les Elbeuf, France). Animals were divided into groups of six per cage and allowed to acclimate for 1 week at 21°C with free access to food and water.

Experimental protocols were implemented according to recommendations of the committee of research and ethics of the International Association for the Study of Pain, and submitted to the local ethical committee. Intrathecal (i.t.) injections were carried out according to the protocol described by Mestre et al. (1994).

Nociceptive Test (Paw Pressure Test). During all the experiments, the evaluation of antinociceptive effects was carried out using the paw pressure test (Randall and Selitto, 1957). Increasing pressures measured with an analgesimeter (tip diameter of the probe, 1 mm; weight, 30 g; Apelex; Ugo Basile, Comerio, Italy) were applied to the left hind paw of rats, with a cutoff at 750 g. Vocalization thresholds, considered nociceptive thresholds, were expressed in grams (baseline predrug vocalization thresholds range from 284 ± 28 g to 316 ± 25 g).

Treatment Protocol

Influence of 5-HT_3 Receptor Antagonists on Acetaminophen-Induced Antinociceptive Effect. We studied the effect of tropisetron (0.5 μg/rat, i.t.), granisetron, and ondansetron (0.1, 1, 10, and 20 μg/rat, i.t.) on the antinociceptive activity of acetaminophen 400 mg/kg (p.o.). All the antagonists were injected 10 min before acetaminophen and were used at doses previously shown to inhibit the antinociceptive action of mCPBG in the same behavioral test in rat (Bardin et al., 1997). Vocalization thresholds were then measured every 15 min for 120 min ($n = 8$ per group) after administration of acetaminophen. Only one dose of tropisetron was used as a control experiment, because its ability to inhibit the effect of acetaminophen has previously been demonstrated (Alloui et al., 1996; Pélissier et al., 1996).

Antisense Experiments. To complete the results obtained with 5-HT_3 receptor antagonists, an antisense approach was used, with AODNs designed to reduce 5-HT_3 receptor synthesis. The base sequence of the 5-HT_3 antisense was adapted from that of an already described antisense oligodeoxynucleotide sequence that efficiently down-regulates mouse 5-HT_3 receptor expression (Paul et al., 2001): CGG GA**T** **G**CA **GA**G CGG CAT. The sequence of the negative control (mismatch) was made by inversion of the two consecutive highlighted bases at two points of the antisense sequence. A blast of the two sequences (http://www.ncbi.nlm.nih.gov/BLAST) enabled us to verify that oligodeoxynucleotides (ODNs) presented no other site of interaction.

Saline (10 μl/rat) and antisense or mismatch ODNs (12.5 μg/rat) were injected i.t. every 12 h for 4 days. Two active doses of mCPBG, determined by a dose-effect relationship study, were administered i.t. on the fifth day of the ODN injection protocol, and their antinociceptive effects were evaluated for 60 min. The same protocol was then applied to assess the antinociceptive activity of acetaminophen (400 mg/kg, p.o.) in rats treated with ODN.

All behavioral experiments were performed blind in a quiet room by a single experimenter using the method of equal blocks with randomization of treatments to avoid any uncontrollable environmental influence that might induce a modification in the behavioral response.

Validation of Antisense Efficiency by Immunoautoradiography. On the fifth day of antisense or mismatch ODN injections, the lumbar enlargement of the spinal cord was removed, and sections from fixed tissues were labeled using the protocol described by Doucet et al. (2000).

All immunolabeling experiments were performed using a purified rabbit polyclonal antibody directed against a 16-amino acid synthetic peptide corresponding to a selective portion of the second intracellular loop of the rat 5-HT_3-A receptor subunit. These antibodies have been shown to specifically interact with the rat 5-HT_3-A receptor subunit (Doucet et al., 2000). Coronal sections were successively incubated with anti-5-HT_{3A} receptor antibodies, and ^{125}I-IgG anti-rabbit IgG. Quantification of ^{125}I-labeling of immunoautoradiograms was made using a Biocom image analyzer.

Drugs. Acetaminophen was from Bristol-Myers Squibb (Rueil-Malmaison, France). Tropisetron was from Sandoz (Rueil-Malmaison, France), and granisetron and ondansetron were from Glaxo-

SmithKline (Marly-Le-Roy, France). mCPBG and serotonin (creatinine sulfate complex) were obtained from Sigma-Aldrich (l'Isle d'Abeau Chesne, France). ODNs were synthesized by Invitrogen (Cergy Pontoise, France). Products administered intrathecally were dissolved in sterile saline (0.9% NaCl; B Braun, Melsungen, Germany).

Expression of Results and Statistical Analyses. Results from electrophysiological and immunoautoradiographic studies are presented as the mean ± S.E.M. and compared using *t* test analysis. Results from behavioral studies are expressed as the difference (in grams) between vocalization thresholds after treatment and control predrug values. To investigate global effects, areas under the time course curves (AUC) of the antinociceptive effects were calculated using the trapezoidal method. Data were analyzed by a two-way analysis of variance followed, when the F-value was significant, by a Dunnett's test to study the time course of the effect or by a one-way analysis of variance to compare the effects of different treatments. Scores were considered significantly different when $p < 0.05$.

Results

In Vitro Electrophysiological Evidence of a Lack of Any Direct Effect of Acetaminophen on 5-HT$_{3A}$ Receptors

Robust expression of homomeric 5-HT$_{3A}$ receptors was obtained in *X. laevis* oocytes as described previously (Maricq et al., 1991). Application of 10 μM 5-HT at different holding potentials revealed the typical functional characteristics of 5-HT$_{3A}$ channels with desensitization current kinetics at negative holding potentials (Fig. 1A) and a linear current-voltage profile with a reversal potential near 0 mV (Fig. 1B). Application of the specific antagonist granisetron (100 nM) blocked completely the 5-HT–evoked responses (data not shown). Homomeric 5-HT$_{3A}$ receptors were equally activated by 5-HT or by the specific agonist mCPBG as shown by the dose-response curves presented in Fig. 1C. In sharp contrast, application of acetaminophen (from 300 μM to 30 mM) was unable to evoke any 5-HT$_{3A}$ receptor activity (Fig. 1C). To confirm the lack of any influence of acetaminophen on 5-HT$_{3A}$ receptors, a potential antagonist activity of acetaminophen at 5-HT and mCPBG sites onto the 5-HT$_{3A}$ receptors was examined. No difference was observed between control and treated groups, indicating that acetaminophen has no 5-HT$_3$ antagonist properties (Fig. 1D).

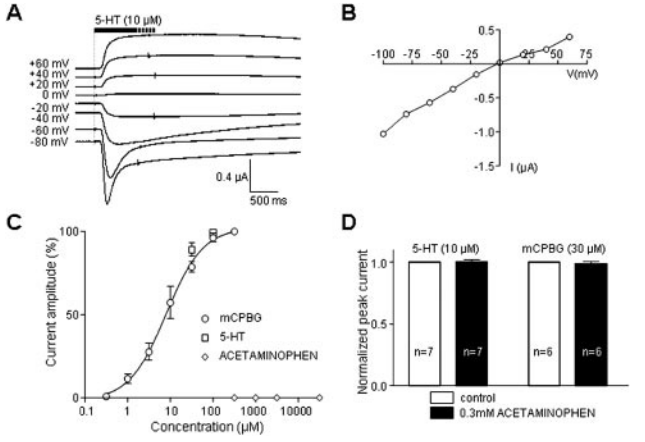

Fig. 1. Acetaminophen has no effect on homomeric 5-HT3A receptors expressed in *X. laevis* oocytes. A, representative traces obtained by the perfusion of 10 μM 5-HT at different membrane potentials. The horizontal bar above the current traces indicates the duration of 5-HT application. B, peak current versus holding potential from the recordings presented in A. C, normalized concentration-response curves (mean ± S.E.M.) recorded at −80 mV for 5-HT (□), mCPBG (○), acetaminophen (◇), (5 to 32). The fitting of the mCPBG curve gives an EC$_{50}$ of 7.8 μM. D, normalized currents evoked by 5-HT (10 μM) or mCPBG (30 μM) in the absence (□) or the presence (■) of 0.3 mM acetaminophen.

Evidence for a Lack of Involvement of 5-HT$_3$ Receptors in the Antinociceptive Effect of Acetaminophen

Acetaminophen administered orally induced a significant and similar antinociceptive effect in all behavioral studies performed.

Influence of 5-HT$_3$ Receptor Antagonists

Tropisetron (0.5 μg, i.t.) totally reversed the antinociceptive effect of acetaminophen. Groups treated with "saline + saline", "tropisetron + saline", or "tropisetron + acetaminophen" presented no significant differences, whereas acetaminophen induced a significant increase in vocalization thresholds compared with the control group (Fig. 2).

Neither ondansetron (Fig. 3, A and B) nor granisetron (Fig. 4, A and B), inactive on their own, significantly altered the antinociceptive effect of acetaminophen at doses up to 10 μg/rat. However, an important yet not significant decrease in AUC scores (−52 ± 20%) was observed after i.t. administration of 20 μg of granisetron. Significant differences were obtained between groups treated with "granisetron + saline" and "granistron + acetaminophen" at 40 and 60 min. For

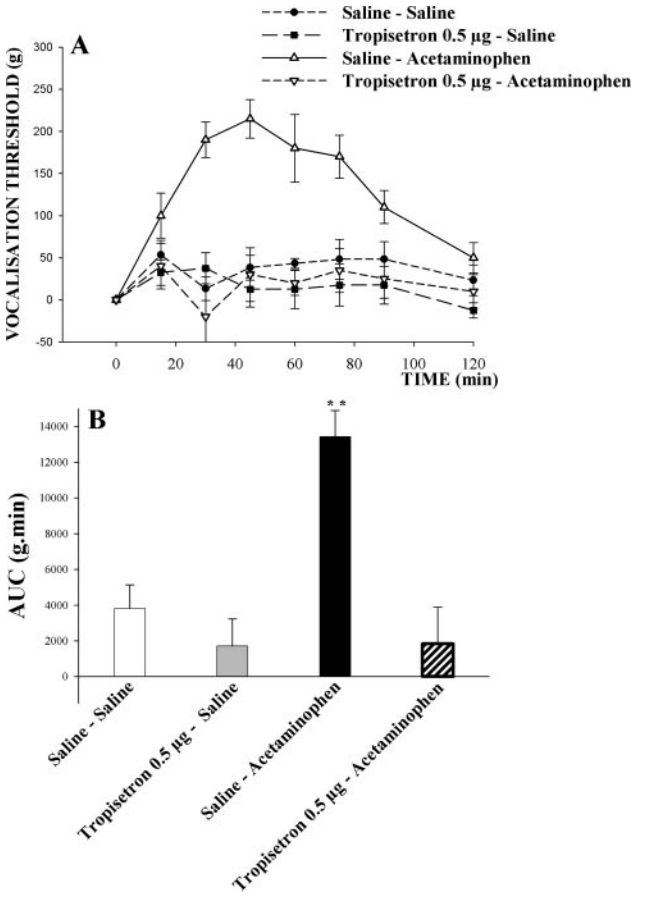

Fig. 2. Influence of tropisetron (0,5 μg, i.t.) on the antinociceptive effect of acetaminophen (400 mg/kg, p.o.) in the paw-pressure test. A, time course of the antinociceptive effect of acetaminophen or saline administered after tropisetron or saline. B, AUC calculated from individual data shown in A. The bars represent the S.E.M. **, $P < 0.01$ versus corresponding scores of (saline + saline) treated group.

ondansetron, the resulting decrease in acetaminophen-induced antinociception was slighter.

Influence of 5-HT$_{3A}$ Receptor-Directed Antisense ODN

Immunoautoradiographic Labeling of Spinal 5-HT$_3$ Receptors in AODN-Pretreated Rats. The labeling is especially intense within the superficial laminae of the dorsal horn, as expected from the known distribution of 5-HT$_3$ receptors in the rat spinal cord (Laporte et al., 1992) (Fig. 5). Compared with saline-treated rats, AODN treatment significantly reduced the optical density in laminae I and II by 29.2 ± 7.9%, whereas mismatch ODN did not significantly change the immunoautoradiographic labeling (Table 1).

Antinociceptive Effect of mCPBG. mCPBG induced a dose-dependent antinociceptive effect that was significant for all the doses tested (Fig. 6). Both intensity (maximal increase, 188 ± 36 g) and duration (maximal, 60 min) of the effect were dose-dependent as confirmed by AUC analysis. The two highest doses (1 and 10 μg/rat, i.t.) were selected to study the influence of AODNs.

Pharmacological Validation of the Efficacy of AODNs. As illustrated in Fig. 7A, it was verified that pretreatment with AODNs significantly attenuated the antinociceptive effect of mCPBG at the two i.t. doses tested. Indeed, AUC analysis showed that, at 1 and 10 μg, the effect of mCPBG was reduced by 58 ± 10% and 60 ± 2%, respectively, in AODN-pretreated rats. On the other hand, mismatch ODNs did not significantly inhibit the effect of mCPBG at 1 μg/rat, i.t. (Fig. 7A).

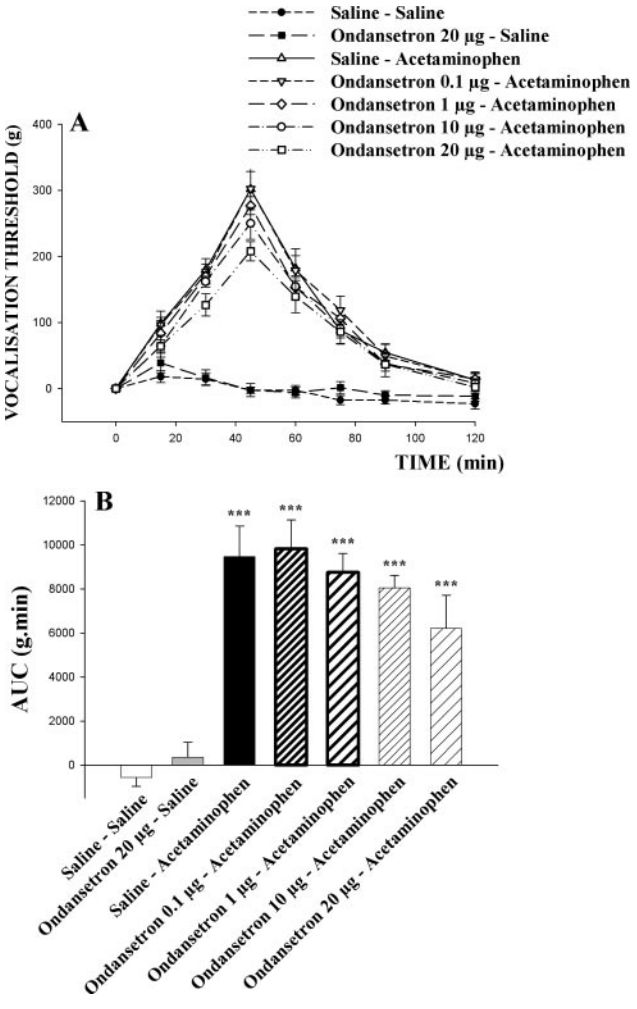

Fig. 3. Influence of ondansetron (0.1, 1, 10, and 20 μg/rat, i.t.) on the antinociceptive effect of acetaminophen (400 mg/kg p.o.) in the paw-pressure test. A, time course of the antinociceptive effect of acetaminophen or saline, administered after ondansetron or saline. B, AUC calculated from individual data shown in A. The bars represent the S.E.M. ***, $P < 0.001$ versus corresponding scores of (saline + saline) treated group.

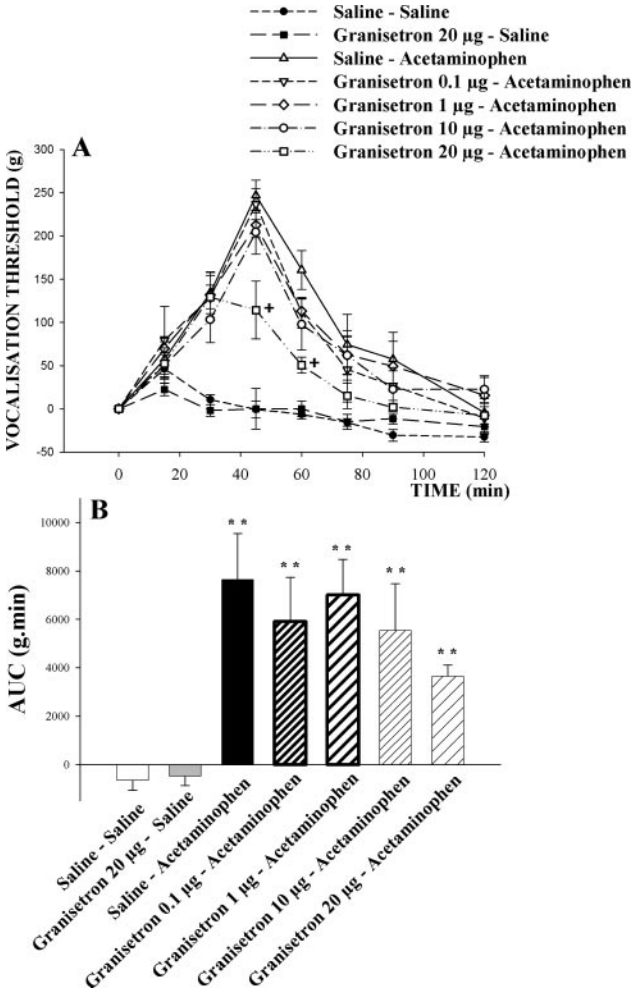

Fig. 4. Influence of granisetron (0.1, 1, 10, and 20 μg/rat, i.t.) on the antinociceptive effect of acetaminophen (400 mg/kg p.o.) in the paw-pressure test. A, time course of the antinociceptive effect of acetaminophen or saline, administered after granisetron or saline. B, AUC calculated from individual data shown in A. Bars represent the S.E.M. +, $P < 0.05$ versus corresponding scores of (acetaminophen + saline) treated group; **, $P < 0.01$ versus corresponding scores of (saline + saline) treated group.

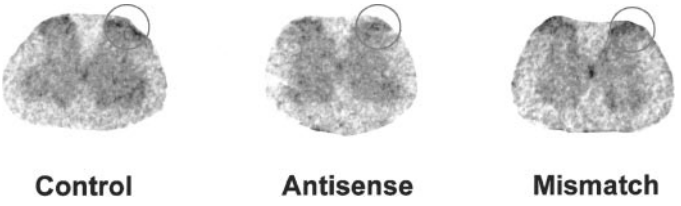

Fig. 5. Immunoautoradiograms of coronal sections at the lumbar level (L4, L5). Animals received eight intrathecal injections of saline (control) or antisense or mismatch ODN for 4 days. Coronal sections were incubated with anti-5-HT$_{3A}$ receptor antibodies and then ^{125}I-IgG anti-rabbit IgG as described under *Materials and Methods*. Immunoautoradiograms are representative of those obtained and quantified in four rats per group (see Table 1).

Effect of AODN Pretreatment on Acetaminophen-Induced Antinociceptive Effect. Pretreatment with ODNs induced no reduction in the antinociceptive effect of acetaminophen (400 mg/kg, p.o.). As illustrated in Fig. 7B, animals treated with acetaminophen had similar vocalization thresholds regardless of the pretreatment (saline, AODNs, or mismatch ODNs) they had received.

Discussion

Two main hypotheses are currently postulated to explain the antinociceptive action of acetaminophen: an inhibition of the cyclooxygenases (although different opinions are presented in relevant literature) or an interaction with the central serotonergic system, as suggested by data collected by different groups (for review, see Bonnefont et al., 2003). In vitro experiments suggest that acetaminophen could inhibit COX-3, a putative new cyclooxygenase isoform more potently inhibited by NSAIDs that also inhibit COX-1 and COX-2 isoforms (Chandrasekharan et al., 2002) in opposition to acetaminophen. However, some reports suggest that either the acetaminophen concentrations needed to inhibit COX-3 are difficult to reach with an oral dose of 0.5 to 1 g (Schwab et al., 2003) or question the existence of the catalytically active form of COX-3 in humans (Dinchuk et al., 2003). The hypothesis of a serotonergic mechanism is based on in vivo experiments involving behavioral assessments (Tjolsen et al., 1991; Pini et al., 1996) and biochemical assays (Srikiatkhachorn et al., 1999; Courade et al., 2001b). In terms of receptors, the 5-HT_3 receptors were suspected to be involved because of the inhibitory effect of tropisetron (Pélissier et al., 1996), but an interaction with other 5-HT receptors was also described previously (Courade et al., 2001c).

Two main results were obtained in this work based on this central serotonergic hypothesis: 1) acetaminophen did not act directly on the 5-HT_{3A} receptors and 2) its antinociceptive effect was inhibited neither by two 5-HT_3 receptor antagonists nor by an AODN-induced down-regulation of a 5-HT_3 receptor in the spinal cord.

TABLE 1

Quantitative immunoautoradiographic labeling of 5-HT_{3A} receptors in rats injected with saline, mismatch or antisense ODN

Quantification of ^{125}I-labeling of immunoautoradiograms at the level of the superficial laminae (I, II) of the dorsal horn was made using a Biocom image analyzer. Optical density values are the mean ± S.E.M. of determination in 24 sections from four rats in each group. Figures in parentheses are the percentage changes relative to controls (saline-treated rats).

Treatment	Optical density (arbitrary units)
Saline	24.05 ± 2.40
Antisense	17.02 ± 2.21* (−29.2%)
Mismatch	22.85 ± 2.30 (−5%)

* $P < 0.05$ compared with saline-treated rats.

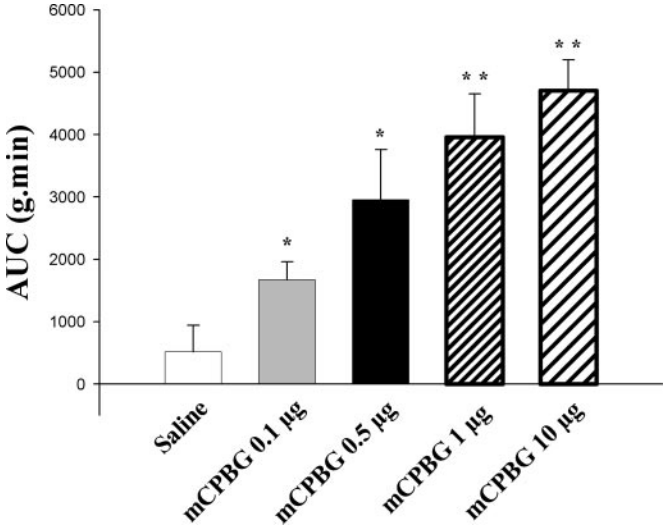

Fig. 6. Antinociceptive effect of mCPBG (0.1, 0.5, 1, 10 μg/rat, i.t.) in the paw-pressure test. A, time course of the antinociceptive effect of mCPBG. B, AUC calculated from individual data shown in A. The bars represent the S.E.M. *, $P < 0.05$, **, $P < 0.01$ versus corresponding scores of saline-treated group.

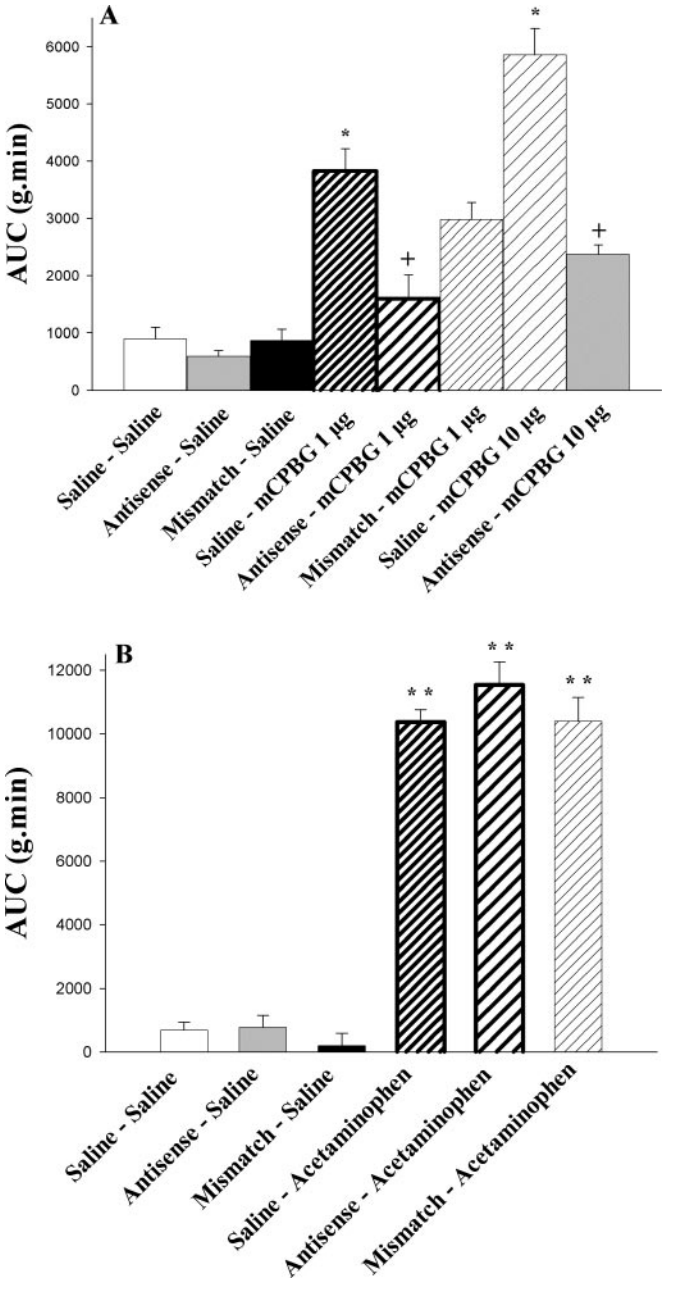

Fig. 7. Influence of antisense or mismatch ODN on the antinociceptive effect of mCPBG (1 and 10 μg/rat, i.t.) (A) and acetaminophen (400 mg/kg, p.o.) (B) in the paw-pressure test. AUC were calculated from individual data of time course curves. The bars represent the S.E.M. *, $P < 0.05$ versus corresponding scores of (saline + saline)-treated group; +, $P < 0.05$ versus scores of corresponding (saline + mCPBG)-treated groups.

Previous studies aimed at identifying possible binding sites for acetaminophen showed a lack of affinity of this drug for 5-HT_3 or other 5-HT receptors (Pélissier et al., 1996; Raffa and Codd, 1996). However, these data were obtained for acetaminophen concentrations up to 10 μM, and one could not exclude some interaction with 5-HT_3 receptors at higher concentrations of the drug. The results obtained in the present electrophysiological studies largely demonstrated the inability of high concentrations of acetaminophen (up to 30 mM) to act on recombinant 5-HT_{3A} receptors. Therefore, these data exclude any direct agonist or antagonist effect of the analgesic drug on 5-HT_{3A} receptors. Two 5-HT_3 receptor subunits have been identified to date (5-HT_{3A} and 5-HT_{3B}), but the rat central nervous system seems to contain only one population of receptors formed by the 5-HT_{3A} subunits only (Morales and Wang, 2002). Therefore, the data obtained using transfected *X. laevis* oocytes probably reflect the situation in the central nervous system, and it can be reasonably concluded that acetaminophen does not interact with 5-HT_3 receptors in the dose range needed for its antinociceptive action. Under these conditions, the demonstrated blockade of the antinociceptive effect of acetaminophen by tropisetron might involve, at best, indirect effects at 5-HT_3 receptors.

In vivo studies confirmed the complete prevention of the antinociceptive effect of acetaminophen by tropisetron but failed to reveal any clear-cut influence of the other two 5-HT_3 receptor antagonists tested, granisetron and ondansetron, up to the doses of 10 μg/rat. At the highest dose used (20 μg/rat), granisetron significantly inhibited the action of acetaminophen 40 and 60 min after its administration. However, granisetron did not modify significantly the effect of acetaminophen on AUC scores. Nor did ondansetron. Furthermore, the specificity of these antagonists at such high doses can be questioned. Previous studies showed, for instance, that 1 μg of granisetron per rat was enough to strongly inhibit, in the same behavioral test, the antinociceptive effect of the potent 5-HT_3 receptor agonist mCPBG (Bardin et al., 1997). In contrast, at this dose, neither granisetron nor ondansetron inhibited acetaminophen-induced antinociceptive effect, whereas tropisetron does at 0.5 and 1 μg/rat (Pélissier et al., 1996). From these data, which show differences between 5-HT_3 receptor antagonists, as previously reported in clinical studies (e.g., Langlois et al., 1996), it can be concluded that 5-HT_3 receptors are not involved in the antinociceptive effect of acetaminophen. It is interesting that Bardin et al. (1997) reached similar conclusions. At doses from 0.001 to 1 μg/rat, tropisetron inhibits the antinociceptive effect of 5-HT in the paw pressure test, whereas granisetron 0.1 and 1 μg/rat does not, supporting a non-5-HT_3–mediated antinociceptive effect of 5-HT.

Tropisetron seems to have specific influences on the antinociceptive effect of acetaminophen and 5-HT. Moreover, it possesses a specific receptor profile; in addition to 5-HT_3 receptor blockade, it binds to 5-HT_4 receptors (Arranz et al., 1998), α7 and α9 nicotinic receptors (Rothlin et al., 1999; Macor et al., 2001), and glycinergic receptors (Maksay et al., 1999). Therefore, the tropisetron-sensitive action of acetaminophen might involve other receptor types than the 5-HT_3 receptors. The antisense study confirmed this hypothesis. The antinociceptive effect of the two highest selected doses of mCPBG, 1 and 10 μg/rat, was reduced by approximately 60% after AODN pretreatment, which induced a 30% reduction of 5-HT_{3A} receptor labeling within the superficial laminae of the dorsal horn of the spinal cord. Such differences between the reduction of receptor density and that of its agonist action have already been described [for example, with opioid receptor (Pasternak and Standifer, 1995)]. The degree of down-regulation of receptors observed with AODNs depends on the sensitivity of the assay system used (for review, see Stone and Vulchanova, 2003). In the hypothesis where 5-HT_3 receptors play a role in the antinociceptive action of acetaminophen, such AODN-induced down-regulation of spinal 5-HT_3 receptors should have affected this drug action. However, after acetaminophen administration, animals pretreated with AODNs presented vocalization thresholds similar to those pretreated with saline or mismatch ODNs. These data further support the conclusion that spinal 5-HT_3 receptors are not involved in the central mechanism underlying the antinociceptive action of acetaminophen.

However, the present results do not exclude the hypothesis of an indirect serotonergic mechanism of action of acetaminophen. This hypothesis is based on previous data evoking a mobilization by acetaminophen of endogenous serotonin (Tjolsen et al., 1991; Pini et al., 1996; Courade et al., 2001b) and showing that 5-HT receptor antagonists similarly influence the antinociceptive effect of 5-HT and that of acetaminophen (Courade et al., 2001c). Therefore, Chen and Bazan (2003) demonstrate an inhibition by acetaminophen of hippocampal synaptic plasticity via a 5-HT_2 receptor and thus suggest the involvement of a release of endogenous 5-HT in this effect. However, the actual effect of acetaminophen on central 5-HT systems remains to be elucidated. In fact, complex interactions between 5-HT neurotransmission and other neurotransmitter systems might be evoked, which would explain the unique ability of tropisetron to prevent the antinociceptive action of acetaminophen. In this respect, particular attention should be focused on cholinergic and glycinergic neurotransmissions, because tropisetron acts at both nicotinic and glycinergic receptors, and these systems are clearly involved in pain modulation (Millan, 2002). Further work is thus needed to clarify the mechanism of the antinociceptive action of acetaminophen. In this context, the present results interestingly demonstrated that this mechanism involves a spinal site of action that can be uniquely affected by tropisetron through non–5-HT_3 receptors.

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