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5-HT receptor subtypes involved in the spinal antinociceptive effect of acetaminophen in rats

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Abstract

The present study was designed to investigate which subtype of spinal 5-HT receptors were involved in acetaminophen-induced antinociception using the paw-pressure test. Propacetamol (prodrug of acetaminophen, 400 mg/kg, injected intravenously, corresponding to 200 mg/kg of acetaminophen) produced a significant antinociceptive effect in this test. This effect was at least partially inhibited by intrathecal (i.t.) pretreatment with the 5-HT_{1B} (penbutolol), 5-HT_{2A} (ketanserin), 5-HT_{2C} (mesulergine) receptor antagonists, but not by the 5-HT_{1A} (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride, WAY 100635) and 5-HT_3 (granisetron) receptor antagonists. This profile was very close to that obtained recently with 5-HT, which suggests an implication of 5-HT in the spinal antinociceptive effect of acetaminophen. These results, the lack of binding of acetaminophen to 5-HT receptors and the increase of central 5-HT levels induced by this drug suggest that acetaminophen-induced antinociception could be indirectly mediated by 5-HT.

Keywords: Acetaminophen; 5-HT receptor antagonist; Antinociception; Paw-pressure test

1. Introduction

Although acetaminophen was discovered more than one century ago (Von Mering, 1893), its mechanism of action remains unclear. It has been suggested that it could exert its analgesic properties at the site of injury. In fact, it was shown to reduce nociception and edema produced by intraplantar injection of carrageenan (Ferreira et al., 1978; Honore et al., 1995). However, anti-inflammatory activity of acetaminophen was a matter of debate (Clissold, 1986). According to Flower and Vane (1972), acetaminophen might be a preferential inhibitor of central more than peripheral cyclooxygenases, but its potency is weak. Warner et al. (1999) have found that concentrations of acetaminophen was 8.5-folds more important than aspirin for inhibiting cyclooxygenese-2 activity (IC_{50} = 64 and 7.5 μM, respectively). In the same study, acetaminophen had no activity on cyclooxygenese-1 activity (IC_{50} > 100 μM). Thus, inhibition of cyclooxygenase is not sufficient to explain its antinociceptive activity.

A growing body of evidence has supported the idea that acetaminophen could act in the central nervous system. Firstly, acetaminophen has been shown to cross the blood–brain barrier. In fact, this drug was recovered in the cerebrospinal fluid (Bannwarth et al., 1992), brain extracellular fluid (De Lange et al., 1994), and brain tissues (Fischer et al., 1981) following its systemic administration. Secondly, in the rodent, intraperitoneal (i.p.) injection of acetaminophen (200–400 mg/kg) reduced the biting, scratching, licking behaviour induced by intrathecal (i.t.) administration of substance P and *N*-methyl-D-aspartate (NMDA) (Björkman et al., 1994), or substance P and capsaicin (Hunskaar et al., 1985). Thirdly, systematically administered acetaminophen (50–150 mg/kg i.p.) reduced thalamic neuronal activity induced by electrical stimulation of the sural nerve in the rat without any inflammation (Carlsson and Jurna, 1987). Finally, acetaminophen demonstrated an antinociceptive action when injected centrally. For example, in the rat, i.t. injection of acetaminophen reduced hyperalgesia (phase 2) observed in the formalin test (Malmberg and Yaksh, 1992).

Serotonin (5-HT) has been proposed to participate in the central antinociceptive effect of acetaminophen. In particular, depletion of serotonin by *p*-chlorophenylalanine

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significantly reduced the antinociceptive effects of i.p. acetaminophen (300 and 400 mg/kg) in the hot plate and formalin tests (Pini et al., 1996), as well as lesion of 5-HT pathways by 5,6-dihydroxytryptamine (Tjølsen et al., 1991). In addition, tropisetron, a 5-HT_3 receptor antagonist, prevented the antinociceptive action of acetaminophen (200 μg i.t. or 200 mg/kg i.v.) in the paw-pressure test (Pélissier et al., 1996). The lack of binding of acetaminophen to 5-HT_3 or other 5-HT receptors ($IC_{50} > 10^{-5}$ M) (Pélissier et al., 1996; Raffa and Codd, 1996) reflects an indirect action. Moreover, acetaminophen was found to increase 5-HT levels in the rat brain (Pini et al., 1996). Thus, the antinociceptive effect of acetaminophen could be the consequence of this increase in 5-HT levels. 5-HT was said to produce a spinal antinociceptive effect in a mechanical pain test (paw-pressure test, Bardin et al., 1997) blocked by 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, and the 5-HT_3 receptor antagonists tropisetron (Bardin et al., 2000). Thus, the aim of the present study was to determine the involvement of these 5-HT and 5-HT_{1A} receptors in the antinociceptive effect of acetaminophen assessed in the same pain test. A similar influence of the tested antagonists on the antinociceptive effect of both acetaminophen and 5-HT would provide a further evidence of the involvement of this monoamine in the action of this widely used drug.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats (200–250 g, Charles River, St-Aubin-lès-Elbeuf, France) were used with the agreement of the local ethical committee in accordance with the National Institutes of Health guide for the care and use of laboratory animals (NIH Publication No. 8023, revised 1978). Upon arrival at the laboratory, they were allowed to acclimate for 1 week, in groups of four rats per cage, with free access to food and water.

2.2. Behavioural procedures

2.2.1. Assessment of nociceptive thresholds

The rats were submitted to the paw-pressure test previously described by Randall and Selitto (1957). Nociceptive thresholds, expressed in grams (g), were measured with a Ugo Basile analgesim (Apelex, tip diameter of the probe, 1 mm; weight, 30 g) by applying an increasing pressure to the left hind paw of rats until a squeak (vocalisation threshold) was obtained (cut-off was 750 g).

2.2.2. Intrathecal injections

Intrathecal injections were performed as previously described (Mestre et al., 1994). Briefly, the rat was held in one hand by the pelvic girdle and a 25-gauge × 1-in. needle connected to a 25-μl Hamilton syringe was inserted into the subarachnoidal space between the spinous processes of L5 and L6, until a tail flick was elicited. The syringe was held in position for a few seconds after the injection of a volume of 10 μl/rat for all the drugs.

2.2.3. Treatment protocol

Tested compounds were administered after the measurement of two consecutive stable vocalisation values. Each 5-HT receptor antagonist (10 μg/rat) was administered i.t. 5 min prior to the intravenous (in a tail vein) injection of propacetamol (400 mg/kg, corresponding to 200 mg/kg of acetaminophen) or vehicle (trisodic citrate, 0.02 g/ml). The thresholds were determined before any injection (control predrug value) and 15, 30, 45, 60, 75, 90, 105 min after the end of the second injection. In each series, several groups ($n = 8$ rats/group) were tested: saline + vehicle, saline + acetaminophen, 5-HT receptor antagonist + saline, 5-HT receptor antagonist + acetaminophen. The dose of 5-HT receptor antagonists (10 μg/rat) was selected on the basis of relevant receptor affinity and selectivity, was sufficient to block the action of selective agonists in nociceptive tests (Bardin et al., 1997; Chojnacka-Wojcik et al., 1994; Giordano, 1991; Gjerstad et al., 1996), and allowed to limit the number of animals used.

The dose of acetaminophen (200 mg/kg, i.v.) has been shown effective on the test used (Pélissier et al., 1996).

All the experiments were performed blind in a quiet room by a single experimenter. Three series of experiments were done. The first was performed to determine the effect of acetaminophen and its interaction with (*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-*N*-(2-pyridinyl) cyclohexanecarboxamide trihydrochloride (WAY 100635), penbutolol, and ketanserin, (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A} receptor antagonists, respectively). The second examined the interactions between acetaminophen and mesulergine, a 5-HT_{2C} receptor antagonist. The last designed interactions between acetaminophen and granisetron, a 5-HT_3 receptor antagonist. For each experimental series, the method of blocks was used. This method consists, in grouping, all randomized treatments in the same lapse of time to avoid any uncontrolled experimental influence. Different animals were used for each experiment.

2.3. Expression of results and statistical analysis

Results were expressed as the difference (in gram) between vocalisation threshold obtained after treatment and control predrug values. To investigate global effects, areas under the time-course curves (AUC) of the antinociceptive effect were calculated using the trapezoidal method. The percentage of inhibition induced by antagonists was calculated from the AUC values using the formula: $((x - y)/x) \times 100$, where x corresponds to the mean score of acetaminophen-induced antinociception when saline was

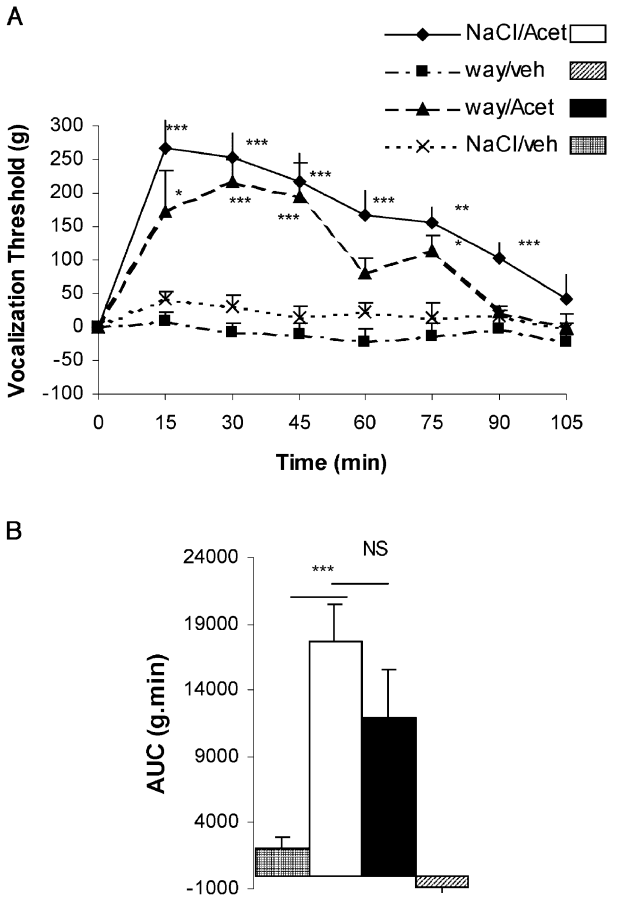

Fig. 1. Influence of i.t. injection of WAY 100635 (way) on the antinociceptive effect of i.v.-administered acetaminophen, assessed by the paw-pressure test. WAY 100635 (10 μg) was given 5 min before acetaminophen (propacetamol: 400 mg/kg corresponding to 200 mg/kg of acetaminophen). Values are the means ± S.E.M. of differences between post- and predrug values of vocalisation thresholds (g). $n = 8$ per group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared with NaCl 0.9% + vehicle (NaCl/Veh)-treated group (A). Areas under the time-course (AUC) of the antinociceptive effect were calculated for each group (B).

used as pretreatment, and y corresponds to the individual score of acetaminophen-induced antinociception when antagonists were used as pretreatments.

Data were analyzed by a two-way analysis of variance (ANOVA), followed, when the F value was significant, by a Dunnett's test, when the time-course of the effects was studied, or by Student's t-test to compare the effect of different treatments when AUC was used. The significant level was $P < 0.05$ for the statistical analysis.

2.4. Drugs

WAY 100635, penbutolol, ketanserin, mesulergine (RBI, France) and Granisetron (Kytril®, Smith-Kline Beecham) were dissolved in physiological saline (0.9% NaCl). Because acetaminophen is weakly soluble in saline, its prodrug propacetamol, Prodafalgan® (400 mg/kg, i.e. 200 mg/kg of acetaminophen; UPSA/BMS, Paris, France), was used for i.v. injections. However, owing to the rapid aqueous hydrolysis of this compound, and to avoid any confusion, we refer only to acetaminophen in the corresponding experiments. Vehicle (trisodic citrate, 0.02 g/ml) was the solvent of propacetamol. Solutions were prepared immediately prior to testing.

3. Results

3.1. Effect of acetaminophen

The mean predrug values of vocalisation threshold were from 217 ± 11 to 299 ± 12 g, according to the experiment. Acetaminophen always induced a significant antinociceptive effect; the maximal vocalisation thresholds were reached 15 min after injection, and varied from 394 ± 40 to 528 ± 58 g, according to the experimental series. The duration of its effect was 90 min, and for one experiment, 75 min (experiment with granisetron).

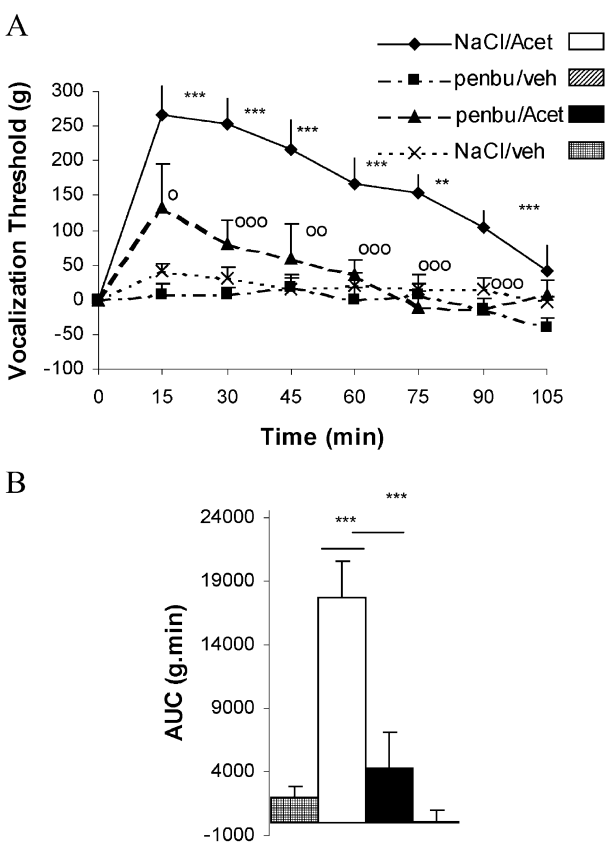

Fig. 2. Influence of i.t. injection of penbutolol (penbu) on the antinociceptive effect of i.v.-administered acetaminophen, assessed by the paw-pressure test. Penbutolol (10 μg) was given 5 min before acetaminophen. For legend, see Fig. 1. $^{o}P < 0.05$, $^{oo}P < 0.01$, $^{ooo}P < 0.001$ as compared with NaCl 0.9% + acetaminophen (NaCl/acet)-treated group.

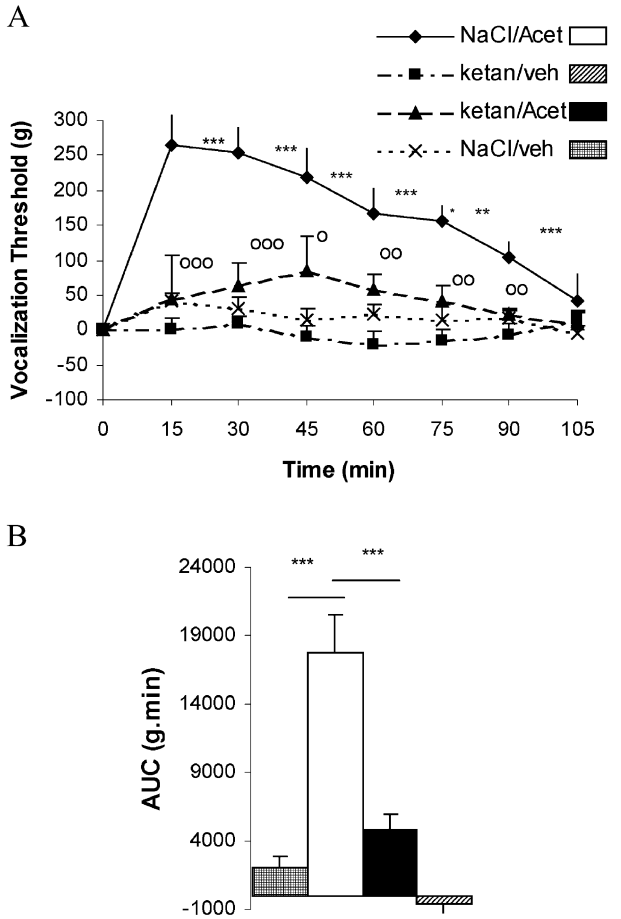

Fig. 3. Influence of i.t. injection of ketanserin (ketan) on the antinociceptive effect of i.v.-administered acetaminophen, assessed by the paw-pressure test. Ketanserin (10 μg) was given 5 min before acetaminophen. For legend, see Fig. 1. $^{o}P < 0.05$, $^{oo}P < 0.01$, $^{ooo}P < 0.001$ as compared with NaCl 0.9% + acetaminophen (NaCl/acet)-treated group.

3.2. Effect of 5-HT$_1$ receptor antagonists on acetaminophen-induced antinociception

The 5-HT$_{1A}$ receptor antagonist WAY 100635 (10 μg/rat) did not significantly alter vocalisation thresholds when administered before the vehicle, nor did it significantly affect the antinociception induced by acetaminophen (Fig. 1).

Similarly, with the same dose, the 5-HT$_{1B}$ receptor antagonist penbutolol did not significantly alter vocalisation thresholds when administered before the vehicle. However, the antinociceptive effect of acetaminophen was significantly inhibited by 10 μg/rat of penbutolol (Fig. 2A). The determination of AUC confirmed the significant inhibition (82 ± 22%) induced by penbutolol (Fig. 2B).

3.3. Effect of 5-HT$_2$ receptor antagonists on acetaminophen-induced antinociception

The 5-HT$_{2A}$ receptor antagonist ketanserin (10 μg/rat) did not significantly alter vocalisation thresholds when administered before the vehicle. The antinociceptive effect of acetaminophen was significantly inhibited by 10 μg/rat of ketanserin throughout the experiment (Fig. 3A). The determination of AUC confirmed this significant inhibition (82 ± 11%) (Fig. 3B).

Intrathecal injection of mesulergine (10 μg/rat), a 5-HT$_{2C}$ receptor antagonist, did not produce any significant effect when administered before the vehicle. However, it significantly inhibited the antinociceptive effect of acetaminophen at 15 and 30 min (Fig. 4A). The determination of AUC confirmed this partial significant inhibition (62 ± 19%) (Fig. 4B).

3.4. Effect of granisetron, a 5-HT$_3$ receptor antagonist, on acetaminophen-induced antinociception

Granisetron, at the doses used (10 μg/rat), did not significantly modify vocalisation thresholds when administered before saline, nor did it significantly affect the antinociception induced by acetaminophen (Fig. 5). How-

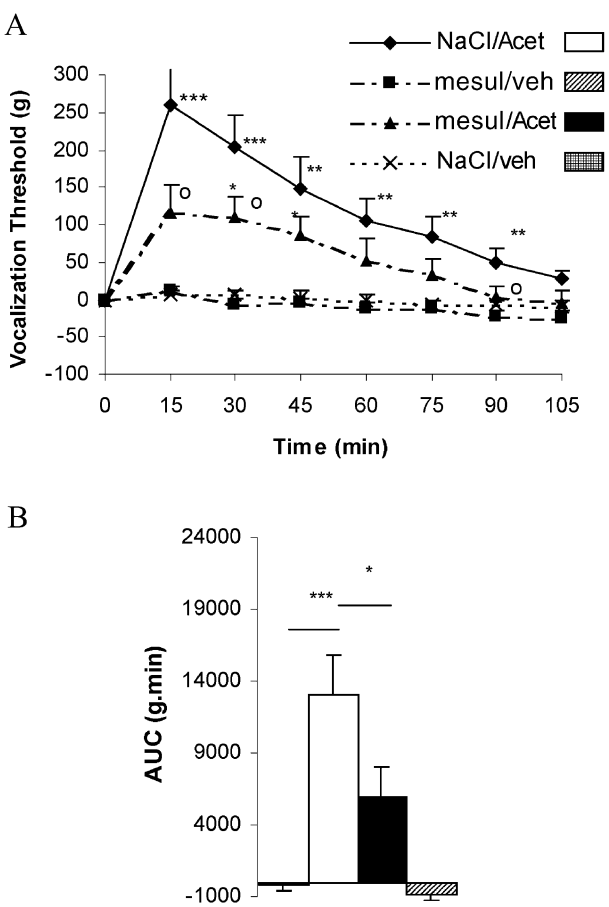

Fig. 4. Influence of i.t. injection of mesulergine (mesul) on the antinociceptive effect of i.v.-administered acetaminophen, assessed by the paw-pressure test. Mesulergine (10 μg) was given 5 min before acetaminophen. For legend, see Fig. 1. $^{o}P < 0.05$, $^{oo}P < 0.01$, $^{ooo}P < 0.001$ as compared with NaCl 0.9% + acetaminophen (NaCl/acet)-treated group.

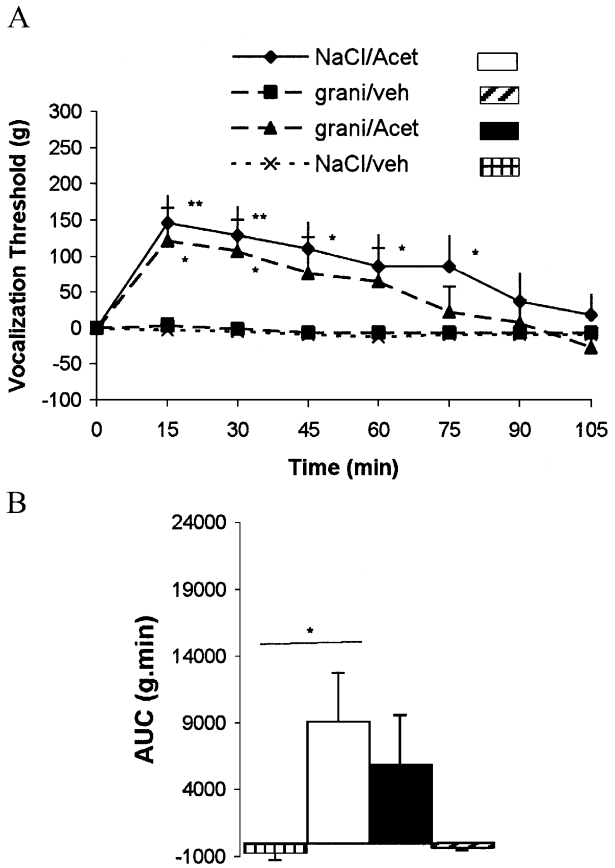

Fig. 5. Influence of i.t. injection of granisetron (grani) on the antinociceptive effect of i.v.-administered acetaminophen, assessed by the paw-pressure test. Granisetron (10 μg) was given 5 min before acetaminophen. For legend, see Fig. 1. The time-course effects of granisetron 10 μg+ vehicle were shown in (B).

ever, it tended to nonsignificantly inhibit the effect of acetaminophen after 10 μg/rat (Fig. 5B).

4. Discussion

The purpose of the present study was to investigate the relative involvement of spinal 5-HT receptor subtypes in the antinociceptive effect of acetaminophen using a mechanical pain test. First of all, we observed, as previously shown (Bardin et al., 1997, 2000), that, in experimental conditions of an acute mechanical pain test, none of the antagonists that were used induce any intrinsic effect. The present findings confirm the lack of any tonic involvement of the serotonergic inhibitory system in these conditions.

Moreover, the present results confirmed the kinetics and magnitude of the antinociceptive effect of intravenously injected acetaminophen in this test, as previously described (Pélissier et al., 1996). The present findings demonstrate the ability of acetaminophen to be effective in conditions of noninflammatory pain, which suggests, once again, a central site of action. Finally, the present work demonstrates a differential involvement of spinal 5-HT receptors.

Among the 5-HT receptor antagonists that were used, only WAY 100635 and granisetron, selective 5-HT_{1A} and 5-HT_3 receptor antagonists, failed to modify the effect of acetaminophen. The involvement of 5-HT_{1A} receptor in pain modulation is a matter of debate (Millan et al., 1996). Its activation at the spinal level induces either antinociceptive (Xu et al., 1994) or pronociceptive action (Alhaider and Wilcox, 1993). However, it is interesting to note that, here, intrathecal injection of WAY 100635 did not change the 5-HT-induced antinociception when using the same test and the same dose (Bardin et al., 2000). The discussion about the involvement of 5-HT_3 receptors, known to play an important role in nociception (Giordano, 1991; Bardin et al., 1997), is more complex. In our study, the i.t. doses of 10 μg/rat of the specific 5-HT_3 receptor antagonist granisetron (Nelson and Thomas, 1989) only slightly diminished acetaminophen antinociception in a nonsignificant manner, whereas it has been shown that the i.t. doses of 10 μg/rat of tropisetron significantly inhibit the effect of intravenously injected acetaminophen (Pélissier et al., 1996). Differences of the effect between these two antagonists have been previously demonstrated. Granisetron was shown to increase the threshold values of noxious colorectal distension in rats pretreated with 5-hydroxytryptophane, whereas tropisetron had no effect (Banner and Sanger, 1995). Newberry et al. (1993) reported, in electrophysiological studies, that tropisetron was slightly more potent than granisetron in inhibiting the 5-HT-induced depolarizing response and that its action reversed more slowly than that of granisetron. Moreover, tropisetron was not very selective for the 5-HT_3 receptor: it binds also to gamma amino butyrique acid A receptor (Klein et al., 1994). In the same test as we used here, while similar i.t. doses of 0.1 to 20 μg/rat of tropisetron and granisetron suppress the antinociceptive effect of *m*-chlorophenyl biguanide, a 5-HT_3 receptor agonist, granisetron (10 μg/rat) only slightly inhibits the 5-HT-induced antinociception, while tropisetron (0.1 to 10 μg/rat) totally suppresses it (Bardin et al., 1997). These effects of granisetron and tropisetron on the 5-HT-induced antinociception are exactly similar to that obtained in the present study and in the study of Pélissier et al. (1996) on acetaminophen. That suggests that this drug could need 5-HT to exert its antinociceptive effect, the antinociceptive action of the monoamine involving a non-5-HT3 receptor more sensitive to tropisetron than to granisetron (Bardin et al., 1997).

On the contrary, the 5-HT_{1B} antagonist penbutolol significantly reduced the acetaminophen-induced antinociception. However, penbutolol is not very selective for the 5-HT_{1B} receptor: it also acts on 5-HT_{1A} receptor β1- and β2-adrenoceptors (Hjorth and Sharp, 1993). However, its effect does not seem to be due to an action on the 5-HT_{1A} receptor since the selective receptor antagonist WAY 100635 had no effect on acetaminophen-induced antinoci-

ception, or on β1- and β2-adrenoceptors, since these receptors do not seem to be implicated in spinal nociception (Yaksh, 1985). Thus, the 5-HT_{1B} receptor is probably implicated in acetaminophen-induced antinociception. Several studies have demonstrated the role of these receptors in the modulation of nociception in the tail flick test (Alhaider and Wilcox, 1993; Xu et al., 1994).

Moreover, penbutolol (10 μg) was found to significantly reduce the 5-HT-induced antinociception using the paw-pressure test (Bardin et al., 2000).

Ketanserin, a 5-HT_{2A} receptor antagonist, also significantly reduced the acetaminophen-induced antinociception. Even though ketanserin also acts on α_1-adrenoceptors and histamine H_1 receptors (Schotte and Leysen, 1988), intrathecal injection of this compound was found to inhibit the antinociceptive effect induced by the 5-HT_2 agonists 1-(2,5-dimethyl-2,4 iodophenyl)-2aminopropane, MK-212 and alpha-methyl-5-HT (Danzbrink and Gebhart, 1991), and the 5-HT-induced antinociception (Bardin et al., 2000). The 5-HT_{2C} receptor antagonist, mesulergine, also significantly reduced the effect of acetaminophen. In fact, this drug was found to block the antinociceptive effect of the 5-HT_{2C} receptor agonist *m*-CPP (Chojnacka-Wojcik et al., 1994), but it also binds to 5-HT_{2A} (Martin and Humphrey, 1994) and 5-HT_7 receptors (Hoyer et al., 1994). Concerning 5-HT_7 receptors, no data are yet available regarding the possible involvement of this receptor in the control of nociceptive messages at the spinal cord level (Vanhoenacker et al., 2000). Mesulergine is not only a 5-HT receptor antagonist, but is also a dopaminergic receptor agonist. However, it is unlikely that the effect of mesulergine occurs via an interaction with dopaminergic receptors because agonists of these receptors have been shown to possess an antinociceptive effect (Fleetwood-Walker et al., 1988). Thus, the influence of mesulergine on the effect of acetaminophen could suggest an involvement of 5-HT_{2C} and 5-HT_{2A} receptors. Taking into account that both its inhibitory effect is only partial (62 ± 19%), and ketanserin, a 5-HT_{2A} receptor antagonist, inhibits the effect of acetaminophen, we can hypothesize that 5-HT_{2A} receptors could be more involved in the effect of acetaminophen than 5-HT_{2C} ones. Nevertheless, whatever the respective involvement of these two receptors are, it is, again, very interesting to observe that, under similar experimental conditions, mesulergine also inhibits 5-HT-induced antinociception (Bardin et al., 2000).

All the data obtained in this study show that several spinal 5-HT receptor subtypes seem to be involved in acetaminophen-induced antinociception, but the major conclusion of this study is that the profile obtained here with acetaminophen is similar to that obtained with the 5-HT (Bardin et al., 1997, 2000). The same doses of the same 5-HT receptor antagonists, intrathecally administered, induce similar reversal or lack of reversal of the antinociceptive effect of 5-HT and acetaminophen. This finding, together with the lack of binding of acetaminophen to 5-HT receptors and uptake sites (Pélissier et al., 1996; Raffa and Codd, 1996), and the fact that this drug increases central 5-HT levels (Pini et al., 1996), led us to postulate that acetaminophen-induced antinociception could be indirectly mediated by 5-HT. Some mechanisms could be proposed concerning the effect of acetaminophen on 5-HT: it could stimulate descending 5-HT inhibitory pathways, or could stimulate 5-HT release at the spinal cord level, or inhibit enzymes implicated in the metabolism of 5-HT. The nature of the molecular mechanism(s) involved in this relationship remains to be elucidated.

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