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The Role of 5-HT$_{1A/B}$ Autoreceptors in the Antinociceptive Effect of Systemic Administration of Acetaminophen

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Background: **It has been proposed that serotonin participates in the central antinociceptive effect of acetaminophen. The serotonin activity in the brainstem is primarily under the control of 5-HT$_{1A}$ somatodendritic receptors, although some data also suggest the involvement of 5-HT$_{1B}$ receptors. In the presence of serotonin, the blockade of 5-HT$_{1A/B}$ receptors at the level of the raphe nuclei leads to an increase in serotonin release in terminal areas, thus improving serotonin functions. This study examines the involvement of 5-HT$_{1A/B}$ receptors in the antinociceptive effect of acetaminophen in mice.**

Methods: **The effects of acetaminophen (600 mg/kg intraperitoneal) followed by different doses of antagonists (WAY 100635 [0.2–0.8 mg/kg subcutaneous] and SB 216641 [0.2–0.8 mg/kg subcutaneous]) or agonists (8-OH-DPAT [0.25–1 mg/kg subcutaneous] and CP 93129 [0.125–0.5 mg/kg subcutaneous]) of 5-HT$_{1A}$ and 5-HT$_{1B}$ receptors, respectively, were determined in the hot-plate test in mice.**

Results: **Acetaminophen (300–800 mg/kg) showed a dose-dependent antinociceptive effect in the hot-plate test in mice. WAY 100635 (0.2–0.8 mg/kg; 5-HT$_{1A}$ antagonist) induced an increase in the antinociceptive effect of 600 mg/kg acetaminophen, but this increase was not dose related. Conversely, 8-OH-DPAT (0.25–1 mg/kg; 5-HT$_{1A}$ agonist) decreased the antinociceptive effect of acetaminophen. SB 216641 (0.2–0.8 mg/kg; 5-HT$_{1B}$ antagonist) induced a dose-related increase in the antinociceptive effect of acetaminophen, and CP 93129 (0.25 mg/kg; 5-HT$_{1B}$ agonist) significantly decreased the antinociceptive effect of acetaminophen.**

Conclusions: **These results suggest that the combination of acetaminophen with compounds having 5-HT$_{1A}$ and 5-HT$_{1B}$ antagonist properties could be a new strategy to improve the analgesia of acetaminophen, thanks to its mild serotonergic properties.**

SINCE its introduction into clinical medicine, acetaminophen (paracetamol) has been used extensively for the treatment of pain. Its therapeutic mechanism of action is still unclear, and it is still not known whether acetaminophen acts peripherally, centrally, or both.[1] It has been proposed that serotonin participates in the central antinociceptive effect of acetaminophen. In support of this, a recent study clearly showed that acetaminophen induces a significant increase in 5-HT levels in the brainstem.[2]

Cell bodies of 5-HT neurons are located almost exclusively in the brainstem, mostly in several midline clusters that lie within or close to the classically defined raphe nuclei. In this area, two nuclei seem to regulate nociception. One of them is the dorsal raphe nucleus, a nucleus that lies within the pons–mesencephalon, and the other is the magnus raphe nucleus, a nucleus that lies within the medulla.[3,4] These nuclei are crucial to the effect of several clinically effective analgesics.[5,6] The serotonin activity in the brainstem, mainly in the dorsal raphe nucleus and the magnus raphe nucleus, is primarily under the control of 5-HT$_{1A}$ somatodendritic receptors,[7] although some data suggest that 5-HT$_{1B}$ receptors[8] also play a role. In the presence of serotonin, the blockade of 5-HT$_{1A}$ receptors at the level of the raphe nuclei leads to an increase in serotonin release in terminal areas,[9,10] thus improving serotonin functions. Moreover, this strategy has also been shown to be effective with the blockade of 5-HT$_{1B}$ autoreceptors in terminal areas.[11,12]

We have previously shown that the antinociceptive effect of tramadol, an analgesic that, like acetaminophen, is able to enhance serotonin levels within the raphe nuclei, is potentiated or antagonized respectively by a 5-HT$_{1A/B}$ nonspecific receptor blockade or activation.[13] More recently, Ardid *et al.*[14] showed that the antinociceptive effect of clomipramine, an inhibitor of 5-HT reuptake, is also enhanced by the specific blockade of 5-HT$_{1A}$ receptors. Thus, there is some evidence that the blockade of 5-HT$_{1A}$ receptors at the level of the raphe nuclei leads to an increase in the antinociceptive effect of some analgesics that increase central 5-HT by different mechanisms (*i.e.*, reuptake inhibition or increased release). Conversely, some observations suggest that stimulation of 5-HT$_{1A}$ receptors reduces the antinociceptive effect induced, for example, by morphine.[15]

In view of the effects of systemic administration of acetaminophen on serotonin levels and activity and previous findings, the purpose of the current study is to evaluate whether the blockade or activation of 5-HT$_{1A}$ or 5-HT$_{1B}$ receptors, following the systemic administration of specific antagonists or agonists, modifies in any way the antinociceptive effect of acetaminophen.

Materials and Methods

Animals

Experiments were performed using albino male mice of the CD1 strain (weight, 25–30 g). All animals were provided by the Servicio de Experimentación y Producción Animal at the University of Cádiz (Cádiz, Spain).

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Table 1. Acetaminophen Effect in Hot Plate Test

	n	Basal Latency	Test Latency	% MPE
Vehicle	10	10.58 ± 0.74	11.71 ± 0.82	3.94 ± 1.34
Acetaminophen	—	—	—	—
300 mg/kg	10	10.56 ± 0.58	17.94 ± 1.36	14.89 ± 2.65
600 mg/kg	10	10.45 ± 0.68	33.37 ± 3.70*	46.11 ± 7.43*
800 mg/kg	10	11.38 ± 0.97	39.75 ± 2.53*	58.76 ± 4.66*

MPE, maximum possible effect.

*$P < 0.05$ *vs.* vehicle

Animals were maintained under standard conditions: 12-h light/dark schedule (lights on at 8:00 h) with *ad libitum* food and water and at constant temperature (21 ± 1°C). The study was conducted according to guidelines for the study of pain in awake animals established by the International Association for the Study of Pain.[16] The experimental protocol was approved by the Local Committee for Animal Experimentation of the Faculty of Medicine at the University of Cádiz (license No. 079604).

Drugs

The following drugs were used: acetaminophen (Sigma, St. Louis, MO), N-2-[4-(2-Methoxyphenyl-1-piperazinyl] ethyl]-N-2-pyridinylcyclohexanecarboxamide (WAY 100635; Sigma), N-3-[3-(Dimethylaminoethoxy]-4-methoxyphenyl]-2'-methyl-4'-(5-methyl-1,2,4-oxadiazol-3-yl)-1,1'-biphenyl-4-carboxamide (SB 216641; Tocris, Bristol, United Kingdom), 8-Hydroxy-2-(di-*n*-propylamine) tetralin (8-OH-DPAT; Sigma), and 1,4-Dihydro-3-(1,2,3,6-tetrahydro-4-pyridinyl)-5H-pyrrolo[3,2-b]pyridin-5-one (CP 93129; Tocris). Control animals received vehicle (30% propylene glycol in distilled water) and saline (0.9% NaCl).

Acetaminophen was suspended in vehicle and intraperitoneally administered at 300, 600, and 800 mg/kg at a volume injection of 20 ml/kg body weight. The other drugs were dissolved in saline and subcutaneously administered in a volume injection of 10 ml/kg body weight. WAY 100635 and SB 216641 were administered at doses of 0.2, 0.4, and 0.8 mg/kg; 8-OH-DPAT was administered at 0.25, 0.5, and 1 mg/kg; and CP 93129 was administered at 0.125, 0.25, and 0.5 mg/kg.

Hot-plate Test

The hot-plate test was performed as described by Woolfe and MacDonald.[17] The hot-plate apparatus (Digital DS-37 Socrel model; Milan, Italy) was thermostatically maintained at 55.5 ± 0.5°C. Animals were placed on the hot plate for each determination, and the latency to the first response, shake or lick of the hind paw or jumping, was recorded as the pain latency in seconds. To avoid tissue damage, a cutoff time was set at 60 s.

Protocol

First, basal latency determination was performed for each animal. The basal latency was calculated as the mean of two determinations with a delay of 30 min between them. After this, acetaminophen or vehicle was intraperitoneally administered, and 15 min later, the antagonist (WAY 100635 or SB 216641) or agonist (8-OH-DPAT or CP 93129) or saline was subcutaneously injected. The hot-plate test was performed 30 min after acetaminophen administration. The researcher who performed the hot-plate test did not know what treatment each animal had received.

Statistical Analysis

Modifications of nociceptive threshold in the hot-plate test were represented by percentages of maximum possible effect (%MPE), which were calculated as:

$$\%\text{MPE} = [(\text{test latency} - \text{basal latency})/(\text{cutoff} - \text{basal latency})] \times 100.$$

The results are expressed as mean ± SEM of %MPE. Statistical analysis was performed using a two-way analysis of variance (ANOVA). The factors of variation were acetaminophen treatment and serotonin antagonist or agonist treatment. Subsequent one-way ANOVA was performed followed by the Student-Newman-Keuls test. A value of $P < 0.05$ was considered to be significant.

Results

Acetaminophen Effect in Hot-plate Test

The antinociceptive effect of acetaminophen was evaluated in the hot-plate test. Basal latencies, test latencies, and %MPE mean values obtained are shown in table 1. One-way ANOVA showed a significant effect of treatment ($F_{3,36} = 30.99$; $P < 0.001$). Acetaminophen induced an increase in pain response latency in a dose-related manner. A dose of 300 mg/kg acetaminophen induced a nonsignificant increase in latency. The antinociceptive effect induced by 600 and 800 mg/kg acetaminophen was statistically significant compared to control animals. We chose the dose of 600 mg/kg acetaminophen to test its association with specific antagonists and agonists of the serotonin receptors subtypes 1A and 1B.

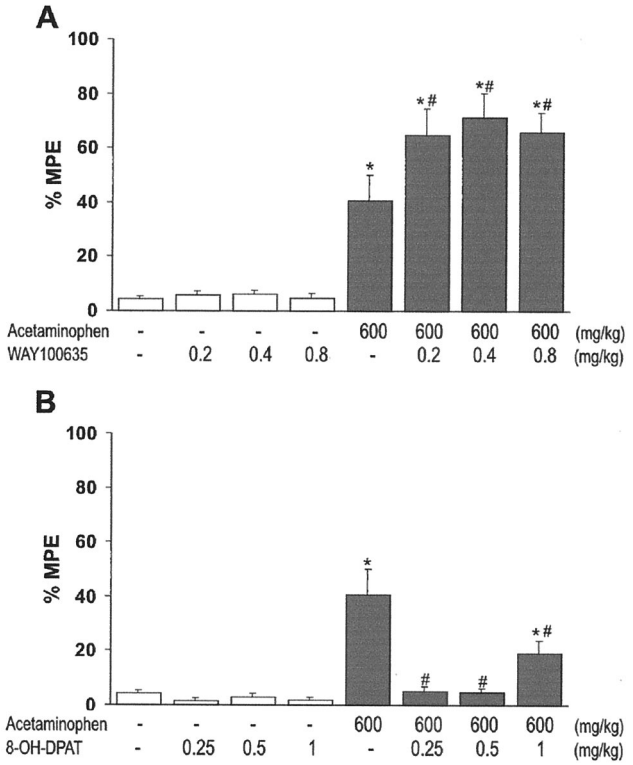

Fig. 1. Implication of the serotonin receptors 5-HT$_{1A}$ on antinociceptive effect of acetaminophen in hot-plate test in mice. The administration of a selective 5-HT$_{1A}$ antagonist WAY 100635 (0.2–0.8 mg/kg subcutaneous; *A*) or a selective 5-HT$_{1A}$ agonist 8-OH-DPAT (0.25–1 mg/kg subcutaneous; *B*) induced a modification on the antinociceptive effect of acetaminophen (600 mg/kg intraperitoneal) in the hot-plate test. Acetaminophen (600 mg/kg) was intraperitoneally injected 30 min before the test. WAY 100635 (*A*) and 8-OH-DPAT (*B*) were administered 15 min after acetaminophen. The figure represents the mean ± SEM of percent maximum possible effect (%MPE); raw data are shown in tables 2 and 3. **P* < 0.05 *versus* vehicle + saline; #*P* < 0.05 *versus* acetaminophen + saline.

Involvement of Serotonin Receptors 5-HT$_{1A}$ in the Antinociceptive Effect of Acetaminophen

Effect of 5-HT$_{1A}$ Antagonist on Antinociceptive Effect of Acetaminophen. Two-way ANOVA revealed a significant effect of acetaminophen ($F_{1,78}$ = 165.22; $P < 0.001$). No significant effect of WAY 100635 (a selective 5-HT$_{1A}$ antagonist; $F_{3,78}$ = 2.73; not significant) was observed, nor any interactions between the two treatments ($F_{3,78}$ = 2.20; not significant). One-way ANOVA showed a significant effect of treatment. WAY 100635 modified the pain latency response in acetaminophen-treated animals ($F_{7,77}$ = 26.02; $P < 0.001$; fig. 1A). Basal latencies, test latencies, and %MPE mean values obtained are shown in table 2. WAY 100635 had no effect in vehicle-treated animals. Mice receiving vehicle with WAY 100635 had a similar latency to that found in the control group.

WAY 100635 induced a significant increase in the antinociceptive effect of acetaminophen, but this increase was not dose-related (table 2).

Effect of 5-HT$_{1A}$ Agonist on Antinociceptive Effect of Acetaminophen. Two-way ANOVA revealed a significant effect of acetaminophen ($F_{1,79}$ = 33.20; $P < 0.001$), 8-OH-DPAT (a specific 5-HT$_{1A}$ agonist; $F_{3,79}$ = 11.87; $P < 0.001$), and interaction between the two treatments ($F_{3,79}$ = 9.68; $P < 0.001$). One-way ANOVA showed a significant effect of treatment ($F_{7,71}$ = 13.31; $P < 0.001$). 8-OH-DPAT modified the antinociceptive effect in acetaminophen-treated animals (fig. 1B). Basal latencies, test latencies, and %MPE mean values obtained are shown in table 3. 8-OH-DPAT did not modify pain response in animals receiving the vehicle (table 3). However, 8-OH-DPAT induced a significant decrease in the antinociceptive effect in acetaminophen-treated animals. This effect was dramatically reduced by doses of 0.25 and 0.5 mg/kg but not by 1 mg/kg, a dose that remained antinociceptive compared to controls.

Involvement of Serotonin Receptors 5-HT$_{1B}$ in the Antinociceptive Effect of Acetaminophen

Effect of Antagonist 5-HT$_{1B}$ on Antinociceptive Effect of Acetaminophen. Two-way ANOVA revealed a significant effect of acetaminophen ($F_{1,79}$ = 129.59; $P < 0.001$), SB 216641 (a selective 5-HT$_{1B}$ antagonist; $F_{3,80}$ = 3.67; $P < 0.01$) and interaction between the two

Table 2. Effect of 5-HT$_{1A}$ Antagonist WAY 100635 on Antinociceptive Effect of Acetaminophen in Hot Plate Test

	n	Basal Latency	Test Latency	% MPE
Vehicle	—	—	—	—
+ Saline	10	10.43 ± 0.74	11.83 ± 1.01	4.18 ± 1.18
+ WAY 100635	—	—	—	—
0.2 mg/kg	10	10.64 ± 0.69	12.92 ± 0.72	5.64 ± 1.56
0.4 mg/kg	10	10.72 ± 0.53	13.65 ± 0.65	6.10 ± 1.34
0.8 mg/kg	10	11.68 ± 0.71	13.62 ± 0.74	4.84 ± 1.59
Acetaminophen, 600 mg/kg	—	—	—	—
+ Saline	9	10.87 ± 0.71	30.54 ± 4.70	40.70 ± 9.31*
+ WAY 100635	—	—	—	—
0.2 mg/kg	9	11.01 ± 0.70	42.37 ± 4.79	64.92 ± 9.44*†
0.4 mg/kg	10	10.01 ± 0.75	45.59 ± 4.51	71.30 ± 8.99*†
0.8 mg/kg	10	10.24 ± 0.64	42.95 ± 3.55	65.68 ± 7.24*†

MPE, maximum possible effect.

*$P < 0.05$ *vs.* vehicle + saline; †$P < 0.05$ *vs.* acetaminophen + saline.

Table 3. Effect of 5-HT$_{1A}$ Agonist 8-OH-DPAT on Antinociceptive Effect of Acetaminophen in Hot Plate Test

	n	Basal Latency	Test Latency	% MPE
Vehicle	—	—	—	—
+ Saline	10	10.43 ± 0.74	11.83 ± 1.01	4.18 ± 1.18
+ 8-OH-DPAT	—	—	—	—
0.25 mg/kg	10	10.87 ± 0.56	10.03 ± 0.85	1.60 ± 0.88
0.5 mg/kg	10	10.97 ± 0.56	11.08 ± 0.94	2.86 ± 1.60
1 mg/kg	10	10.04 ± 0.53	9.41 ± 0.94	1.82 ± 1.13
Acetaminophen, 600 mg/kg	—	—	—	—
+ Saline	9	10.87 ± 0.71	30.54 ± 4.70	40.70 ± 9.31*
+ 8-OH-DPAT	—	—	—	—
0.25 mg/kg	9	10.79 ± 0.60	12.90 ± 0.98	5.06 ± 1.81†
0.5 mg/kg	10	12.39 ± 0.96	12.38 ± 0.90	4.64 ± 1.47†
1 mg/kg	10	10.47 ± 0.36	19.56 ± 2.57	19.07 ± 4.61*†

MPE, maximum possible effect.

*$P < 0.05$ *vs.* vehicle + saline; †$P < 0.05$ *vs.* acetaminophen + saline.

treatments ($F_{3,80} = 3.50$; $P < 0.02$). One-way ANOVA showed a significant effect of treatment ($F_{7,78} = 21.84$; $P < 0.001$). SB 216641 modified the antinociceptive effect in acetaminophen-treated animals (fig. 2A). Basal latencies, test latencies and %MPE mean values obtained are shown in table 4. SB 216641 did not modify the pain response in animals receiving vehicle (table 4). SB 216641 induced a dose-related increase in the antinociceptive effect of acetaminophen. These increases were statistically significant only at doses of 0.4 and 0.8 mg/kg when compared to animals receiving acetaminophen and saline.

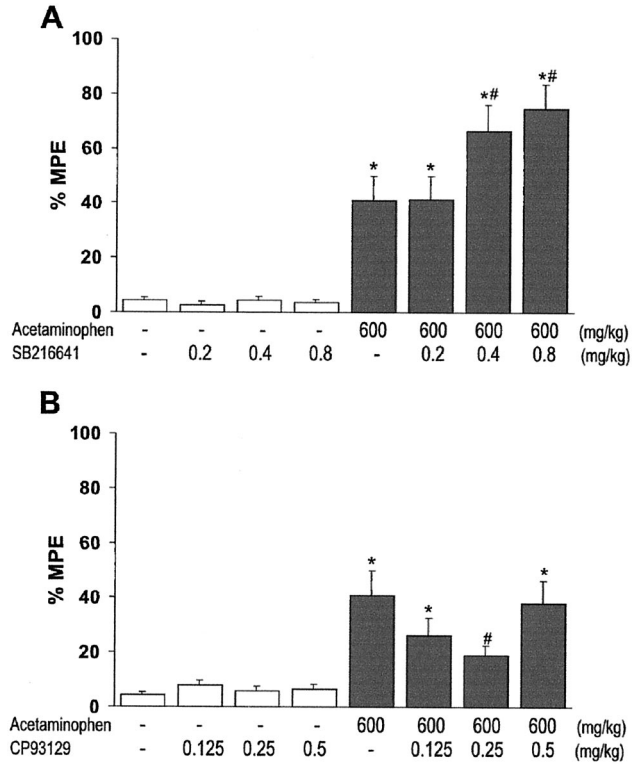

Fig. 2. Involvement of serotonin receptors 5-HT$_{1B}$ on antinociceptive effect of acetaminophen in hot-plate test in mice. The injection of the selective 5-HT$_{1B}$ antagonist SB 216641 (0.2–0.8 mg/kg subcutaneous; *A*) or the selective 5-HT$_{1B}$ agonist CP 93129 (0.125–0.5 mg/kg; *B*) induced a modification on the antinociceptive effect of acetaminophen (600 mg/kg intraperitoneal). Acetaminophen (600 mg/kg) was intraperitoneally administered 30 min before the test. SB 216641 (*A*) and CP 93129 (*B*) were administered 15 min after acetaminophen. The figure shows the mean ± SEM of percent maximum possible effect (%MPE); raw data are expressed in tables 4 and 5. **P* < 0.05 *versus* vehicle + saline; #*P* < 0.05 *versus* acetaminophen + saline.

Effect of 5-HT$_{1B}$ Agonist on Antinociceptive Effect of Acetaminophen. Two-way ANOVA revealed a significant effect of acetaminophen ($F_{1,78} = 46.87$; $P < 0.001$). No significant effect of CP 93129 (a selective 5-HT$_{1B}$ agonist) was observed ($F_{3,78} = 1.70$; not significant). No significant interactions between the two treatments were noted ($F_{3,78} = 2.26$; not significant). One-way ANOVA showed a significant effect of treatment ($F_{7,72} = 8.40$; $P < 0.001$). CP 93129 modified the pain latency response in acetaminophen-treated animals (fig. 2B). Basal latencies, test latencies, and %MPE mean values obtained are shown in table 5. CP 93129 had no effect on pain response in vehicle-treated animals compared to saline-treated animals (table 5). CP 93129 induced a significant decrease in pain response latency in acetaminophen-treated mice. This reduction was significant only at a dose of 0.25 mg/kg when compared to acetaminophen. However, the antinociceptive effect of acetaminophen remained in all groups of treatment compared to vehicle-saline–treated animals.

Discussion

This is the first report of acute intraperitoneal administration of acetaminophen having an antinociceptive effect in mice subjected to the hot-plate (55°C) test. In rats, the antinociceptive effect of acetaminophen on the hot-plate test (54 ± 0.4°C) was demonstrated at a dose 400 mg/kg intraperitoneal,[18–20] while lower doses have no antinociceptive effect.[20,21] In mice, an antinociceptive effect in this test has not been reported until now, owing to the low doses (50–125 mg/kg) used in these

Table 4. Effect of 5-HT_{1B} Antagonist SB216641 on the Antinociceptive Effect of Acetaminophen in Hot Plate Test

	n	Basal Latency	Test Latency	% MPE
Vehicle	—	—	—	—
+ Saline	10	10.43 ± 0.74	11.83 ± 1.01	4.18 ± 1.18
+ SB 216641	—	—	—	—
0.2 mg/kg	10	11.82 ± 0.45	12.22 ± 0.58	2.48 ± 1.30
0.4 mg/kg	10	10.74 ± 0.90	12.25 ± 1.29	4.13 ± 1.66
0.8 mg/kg	10	9.94 ± 0.40	10.67 ± 0.80	3.46 ± 1.33
Acetaminophen, 600 mg/kg	—	—	—	—
+ Saline	9	10.87 ± 0.71	30.54 ± 4.70	40.70 ± 9.31*
+ SB 216641	—	—	—	—
0.2 mg/kg	10	12.24 ± 0.45	31.94 ± 4.17	41.24 ± 8.75*
0.4 mg/kg	10	11.93 ± 0.76	43.66 ± 4.72	66.48 ± 9.53*†
0.8 mg/kg	10	12.10 ± 0.55	47.87 ± 4.26	74.41 ± 9.07*†

MPE, maximum possible effect.

*$P < 0.05$ *vs.* vehicle + saline; †$P < 0.05$ *vs.* acetaminophen + saline.

experiments.[22,23] In the current study, acetaminophen had an antinociceptive effect at 600 and 800 mg/kg intraperitoneal but not at 300 mg/kg, a dose higher than that used previously.[22,23]

A close relation between the 5-HT system and acetaminophen has been demonstrated in several previous studies. In serotonin-lesioned rats, the antinociceptive effect of acetaminophen was significantly reduced.[24] Moreover, depletion of brain serotonin with *p*-chlorophenylalanine, a tryptophan hydroxylase inhibitor, also prevents the antinociceptive effect of acetaminophen and at the same time reduces the 5-HT content in cortical and pontine areas induced by acetaminophen.[18] On the other hand, acetaminophen induces a down-regulation of 5-HT_{2A} in the frontal cortex in response to 5-HT release.[25]

In agreement with the findings cited above, the main result of the current study is that the antinociceptive effect of acute systemic administration of acetaminophen in the hot-plate test in mice is increased both by the selective blockade of 5-HT_{1A} receptors with WAY 100635 and by the selective blockade of 5-HT_{1B} receptors with SB 216641, both administered systemically. Conversely, subcutaneous administration of agonists selective for these receptors antagonized the analgesic effect of acetaminophen. These agonists and antagonists of 5-HT_1 receptors have been demonstrated to be selective ligands of these receptors and to exert these pharmacological actions *in vivo* (for review, see Stamford *et al.*[8]).

Similar results have been obtained with the analgesic tramadol,[13] the analgesic effect of morphine in combination with clomipramine and the antinociceptive and antidepressive effect of clomipramine.[14] With tramadol, racemic pindolol was used as the antagonist, whereas Ardid *et al.*[14] used WAY 100635. Recent studies and others in progress in our laboratory (unpublished observations, Juan A. Micó, Ph.D., Cádiz, Spain; WAY 100635 induces an increase in the antinociceptive effect of tramadol, an analgesic with serotonergic properties) also show that systemic administration of WAY 100635 is able to enhance the analgesic effect of tramadol.

This strategy of potentiation was primarily thought to enhance the effectiveness of antidepressants that inhibit the reuptake of serotonin[12,26,27] and that have proven effective in depressed patients.[14,28,29] However, as has been shown previously and is demonstrated here with acetaminophen, this pharmacological combination might

Table 5. Effect of 5-HT1B Agonist CP93129 on the Antinociceptive Effect of Acetaminophen in Hot Plate Test

	n	Basal Latency	Test Latency	% MPE
Vehicle	—	—	—	—
+ Saline	10	10.43 ± 0.74	11.83 ± 1.01	4.18 ± 1.18
+ CP 93129	—	—	—	—
0.125 mg/kg	10	11.33 ± 0.63	15.00 ± 0.77	8.04 ± 1.46
0.25 mg/kg	10	12.01 ± 0.50	13.51 ± 1.30	5.80 ± 1.89
0.5 mg/kg	10	11.31 ± 0.49	14.21 ± 1.00	6.41 ± 1.67
Acetaminophen, 600 mg/kg	—	—	—	—
+ Saline	9	10.87 ± 0.71	30.54 ± 4.70	40.70 ± 9.31*
+ CP 93129	—	—	—	—
0.125 mg/kg	10	11.42 ± 0.84	24.22 ± 3.05	26.15 ± 6.40*
0.25 mg/kg	9	9.89 ± 0.67	19.49 ± 1.72	19.06 ± 3.56†
0.5 mg/kg	10	10.62 ± 0.54	29.35 ± 4.09	38.03 ± 8.34*

MPE, maximum possible effect.

*$P < 0.05$ *vs.* vehicle + saline; †$P < 0.05$ *vs.* acetaminophen + saline.

also enhance the effect of analgesics with mild serotonergic properties.

The pharmacological mechanism of this combination strategy has been extensively studied with antidepressants.[29,30] In fact, a large body of evidence suggests that acute administration of several compounds, such as selective serotonin reuptake inhibitor, clomipramine, morphine, and tramadol (and probably acetaminophen), leads to an increase in the extracellular 5-HT concentrations in the vicinity of the cell body and the dendrites of 5-HT neurons of the raphe nuclei. Thus, the possibly limited acute effects of these compounds on extracellular 5-HT concentrations could be because of the action of these full or partial serotonergic agents indirectly activating somatodendritic 5-HT_{1A} autoreceptors in the raphe region. The 5-HT excess induced by the specific action of these compounds might activate the somatodendritic 5-HT_{1A} autoreceptors. A negative feedback control might thus be brought into play, leading to the hypoactivity of these neurons, *i.e.*, a decrease in the electrical activity of raphe neurons, as well as reduced 5-HT synthesis and release by nerve endings. Therefore, the pharmacological efficacy (antidepressive and/or analgesic) of these compounds could be limited by this negative feedback. In accordance with this interpretation, the preventive blocking of somatodendritic 5-HT_{1A} by specific antagonists prevents the reduction of 5-HT release in terminal areas and potentiates their effects.[31,32]

Furthermore, our results show that the analgesic effect of acetaminophen is potentiated by the systemic administration of SB 216641, a selective 5-HT_{1B} antagonist, and is antagonized by CP 93129, a selective 5-HT_{1B} agonist. To our knowledge, this is the first observation of the facilitation of the effect of an analgesic with a 5-HT_{1B} receptor antagonist. We have previously shown that racemic pindolol enhances the analgesic effect of tramadol, but that pindolol also blocks 5-HT_{1A} receptors and is a partial agonist of the β-adrenergic receptors.[33] The presence of 5-HT_{1B} receptors in the raphe nuclei is a topical issue (for review, see Stamford *et al.*[8]). This receptor does not control raphe nuclei 5-HT cell firing but appears to regulate 5-HT release within the raphe nuclei where their location is unclear, *i.e.*, either on dendrites or on recurrent collaterals.[34] However, there is evidence that 5-HT_{1B} receptors are located on 5-HT nerve terminals in many central nervous system regions.[35] This receptor has consistently been implicated in pain processes, but at the spinal level.[36] This difference in the location and function of 5-HT_{1A} and 5-HT_{1B} receptors likely accounts for the differences observed in the effects induced by WAY 100635 and SB 216641. In fact, whereas the facilitation induced by SB 216641 seemed to be dose related, a ceiling effect was observed with WAY 100635. Moreover, the inhibition induced by the 5-HT_{1A} and 5-HT_{1B} agonists was not dose dependent in any of the cases, and at higher doses, both agonists showed a tendency for their effects to be reduced, reflecting a probable postsynaptic location of these receptors in terminal areas.[35]

Recently, Courade *et al.*[37] have shown that the antinociceptive effect of acetaminophen is not modified in any direction by intrathecal pretreatment with WAY 100635 but that it is partially inhibited by intrathecal penbutolol, a nonspecific 5-HT_{1B} antagonist.[38] Several differences between these two studies make them particularly difficult to compare. First and most importantly, the administration route and doses of WAY 100635 and the type of 5-HT_{1B} antagonist used in each study were different. Moreover, the nociceptive test and animal species were not the same. However, an interesting observation was that high doses of CP 93129 did not antagonize the effect of acetaminophen and that the highest dose of 8-OH-DPAT used was less effective than the lowest. Taking these results together with those obtained by Courade *et al.*,[37] we suggest that the different locations of 5-HT_{1A} and 5-HT_{1B} at the level of the raphe nuclei or the spinal level might be important regarding the serotonergic mechanisms of acetaminophen. It is important to note that *in vitro* acetaminophen does not show affinity to any serotonergic receptors, at least up to 10 μM.[39] Thus, the effect of acetaminophen on the serotonergic pathway must be indirect.

In conclusion, these findings reinforce the evidence for a central component of action of acetaminophen involving the serotonergic pathways. In addition, given that some nonsteroidal antiinflammatory drugs are able to enhance serotonergic transmission in a similar way to acetaminophen,[40] it would be interesting to explore whether the facilitation of the antinociceptive effects induced by 5-HT_{1A} and 5HT_{1B} antagonists with acetaminophen could be reproduced with these other analgesics. If this is the case, a new analgesic strategy could be developed with this combination, as is the case in other clinical situations.[41–43]

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