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Differential involvement of central 5-HT_{1B} and 5-HT_3 receptor subtypes in the antinociceptive effect of paracetamol

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Abstract. *Objective*: We investigated the effect of pre-treatment with ondansetron or CP 93129 (a 5-HT_{1B} agonist) on the antinociceptive activity of paracetamol and the changes in central 5-HT_3 receptors induced by paracetamol alone or co-administered with ondansetron.
Materials and Subjects: Male Wistar rats (eight per group) were injected with ondansetron (2 and 4 mg/kg s.c.) or CP 93129 (0.5, 1 and 2 mg/kg s.c.) 15 min before paracetamol (400 mg/kg, i.p.).
Methods: Pain threshold was evaluated in the hot-plate or in the paw pressure test 30 min after the last treatment. 5-HT_3 receptor binding capacity was measured in the frontal cortex, temporal-parietal cortex and midbrain by means of radioligand binding technique. Statistical analysis was done using ANOVA followed by Student-Newman-Keuls test and 2 X 2 factorial analysis when appropriate.
Results: Pre-treatment with ondansetron, at doses of 2 and 4 mg/kg, did not affect the antinociceptive activity of paracetamol in the hot-plate test and in the paw pressure test. Paracetamol did not change the characteristics of 5-HT_3 receptors in all the areas investigated. Ondansetron (4 mg/kg s.c) per se significantly increased the 5-HT_3 receptor number in the areas used, the effect not being modified by co-administration with paracetamol. On the other hand, CP 93129 (2 mg/kg s.c.) significantly prevented the effect of paracetamol in both algesimetric tests used.
Conclusions: Our data indicate that 5-HT_{1B} but not 5-HT_3 receptors are involved in the antinociceptive effect of paracetamol in our experimental conditions.

Keywords: Paracetamol – Antinociception – 5-HT_{1B} and 5-HT_3 receptors – Ondansetron – CP 93129 – Brain

Introduction

Many reports indicate that paracetamol exerts its antinociceptive activity acting not only peripherally, but also within the central nervous system (CNS) both at spinal and supraspinal level [1–3]. It has been suggested that a number of neurotransmitter systems may be involved in the central analgesic activity of paracetamol; in particular serotonin (5-HT) pathways seem to play a pivotal role in the central mechanism of action of this drug [4–6]. In our previous works we have demonstrated the importance of the central serotonergic system in the antinociceptive effect of paracetamol which matches an increase in the serotonin levels and a decrease in the number of 5-HT_2 receptors in the cerebral cortex of the rat [6].

Indeed, multiple serotonin subtypes have been identified within the central nervous system and 5-HT_1, 5-HT_2 and 5-HT_3 receptors are now thought to be involved in the 5-HT-mediated antinociceptive mechanism [7]. There are conflicting findings concerning the relationship between the antinociceptive effects of 5-HT to specific subtypes of 5-HT receptors; recently it has been suggested that 5-HT_2 and 5-HT_3 receptors mediate antinociception to chemical stimuli in the spinal cord [8]. Some reports indicate that, at spinal level, 5-HT_3 receptors have an important role in the analgesic effect of paracetamol [9]. 5-HT_3 receptors are concentrated in the dorsal horn of the spinal cord [10], but are present also in some CNS areas, chiefly in the hippocampus, limbic and cortical areas [11].

5-HT_{1B} receptors have been recently proposed to be involved in the antinociceptive action of some drugs. It has been reported that 5-HT_{1B} receptors are predominantly located on nerve terminals [12] where they regulate neurotransmitter release. Activation of 5-HT_{1B} autoreceptors on serotonergic terminals resulted in inhibition of serotonin release from hippocampus and frontal cortex in rats [13]. It has also been shown that 5-HT_{1B} receptors work as heteroreceptors to inhibit the release of other neurotransmitters (glutamate, GABA and acetylcholine) [14]. 5-HT_{1B} receptors are present in the dorsal root ganglia, spinal cord and trigeminal nucleus [15], but also in many CNS regions, including the frontal cortex [16].

Therefore, in the light of the findings that 5-HT_3 receptors might be involved in the mechanism of the antinociceptive action of paracetamol at spinal level, and that they are

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present also in higher brain centres, we decided to evaluate the role of these serotonergic receptors at supraspinal level as well. This we did by studying the influence of the pre-treatment with ondansetron, a specific antagonist of 5-HT_3 receptors, on the antinociceptive effect of paracetamol, evaluated by means of the hot-plate and of the paw pressure tests.

Moreover, since it has been shown that 5-HT_{1B} receptor antagonists increased basal levels of serotonin while their activation decreased serotonin levels [17], we decided to test the influence of CP 93129, a 5HT_{1B} receptor agonist, on the antinociception exerted by paracetamol on the two above mentioned tests.

To exclude the 5-HT_3 receptors' involvement in the antinociceptive effect of paracetamol from a biochemical point of view, we investigated the capability of paracetamol to bind to or to modify the characteristics of 5-HT_3 receptors in three brain areas. Moreover, we studied the influence of pre-treatment with ondansetron on the possible changes induced by paracetamol in the characteristics of brain 5-HT_3 receptors.

Materials and methods

Animals

Male Wistar rats weighing 180–200 g were housed in Plexiglas® cages in groups of three in controlled conditions (free access to food and water; 12-h dark/light cycle; temperature, 22 ± 1 °C; humidity, 60%). The ethical guidelines for investigation of experimental pain in conscious animals were followed, and procedures were carried out according EEC ethical regulations for animal research (EEC Council 86/609; D.L. 27/01/1982, No. 116).

Drug treatment

The rats were randomly divided into groups of eight animals each.

Ondansetron, dissolved in saline, at dosages of 2 and 4 mg/kg s.c. or saline were injected 15 min before paracetamol (400 mg/kg i.p.) (dissolved in vehicle, which consisted of 12.5% of 1,2-propanediol in sterile saline) or vehicle and the animals were subjected to the hot-plate test or to paw pressure test 30 min later. This dose of paracetamol was scheduled as being effective in our experimental conditions on the basis of dose-response experiments in the test performed in our laboratory under identical experimental conditions [6]; the used doses of ondansetron have been selected in the range of those proved to be effective in preventing the antinociceptive activity of acetylsalicylic acid [18].

CP 93129 or saline was injected at the doses of 0.5, 1 and 2 mg/kg s.c. 15 min before paracetamol or vehicle; the rats were subject to the above described behavioural tests following the same experimental schedule.

Control experiments showed no significant difference in response to saline and 12.5% of 1,2-propanediol in the concentration used, so the data have been pooled. Control rats, paracetamol-, ondansetron-(4 mg/kg) and ondansetron plus paracetamol-treated rats were anaesthetized and decapitated immediately after the hot-plate test; their brains were removed and areas dissected and stored at –80 °C until required for assay.

Hot-plate test

The hot-plate consisted of an electrically-heated surface (Socrel DS-35, Ugo Basile, Comerio, VA, Italy) kept at a constant temperature of 54 ± 0.4 °C. The latencies for paw licking or jumping were recorded for each animal. The analgesic efficacy of the drug was evaluated as a percentage of the maximum possible effect (% MPE), according to the formula (TL−BL)/(45−BL) × 100, where TL = Test Latency, BL = Baseline Latency, 45 = cut-off time, in seconds.

Paw pressure test

The rats were submitted to the paw pressure test previously described by Randall and Selitto [19]. Nociceptive threshold, expressed in grams, was measured with an algesimeter (Apelex tipe; tip diameter of probe 1 mm) from Ugo Basile (Comerio, VA, Italy), applying increasing pressure (15 g per sec) in the left paw of rats until the paw withdrawal was obtained after a sort of struggle. Results were expressed as a percentage of the maximum possible effect (% MPE), according to the formula (TL−BL)/(750−BL) × 100, where TL = Test Latency (in grams), BL = Baseline Latency (in grams), cut-off value: 750 g.

Binding assay

The characteristics of 5-HT_3 binding sites were evaluated according to Kilpatrick and co-workers [20] with minor modifications, using six concentrations of [^{3}H]GR65630 (0.1–4 nM; specific activity: 80 Ci/mmol). Frontal cortex, temporal-parietal cortex and midbrain were homogenized in 10 ml of HEPES buffer (50 mM, pH 7.4, 4 °C) (Ultra Turrax, 15 sec) and centrifuged for 10 min at 48000 × g in a Beckman centrifuge at 4 °C. The supernatant was discharged, the pellet was resuspended and, after homogenisation in the same buffer, centrifuged again.

Aliquots of the membrane suspension were used for saturation studies. Non specific binding was determined by metoclopramide (50 µM). The mixture of membranes, paracetamol and HEPES or metoclopramide was incubated at 37 °C for 30 min and the assay was stopped by filtration under reduced pressure through GF/B filters, which had been pre-soaked in 0.1% polyethyleneimine and rinsed with 5 × 3 ml of HEPES at room temperature. Filters were transferred into plastic vials containing 6 ml of Packard Optiluor. The vials were stored overnight at room temperature before being counted.

Competition binding experiments used 10 concentrations between 0.1 nM and 100 µM unlabelled paracetamol to displace binding of 1 nM [^{3}H]GR65630. Binding assay was then performed according to the above method.

Drugs

Paracetamol, metoclopramide and HEPES were purchased from Sigma-Aldrich Srl (Milan, Italy); ondansetron was kindly provided by Glaxo Ltd (Verona, Italy); CP 93129 was purchased by Tocris Cookson Ltd. (Bristol, Great Britain); [^{3}H]GR65630 (specific activity, 80 Ci/mmol) was from Du Pont NEN (Milan, Italy).

Data analysis

The results of binding experiments were analysed according to the method of Rosenthal. The equilibrium dissociation constant (k_D) and the maximum number of binding sites (B_{max},) were evaluated individually for each sample with six concentrations of labelled drug using the regression analysis; binding parameters were calculated from the plot [21].

The data obtained from the hot-plate and paw pression tests and from binding experiments were expressed as means ± SEM and correlated by using one-way analysis of variance (ANOVA) followed, when F-value was significant, by the Student-Newman-Keuls test in case that the effects of ondansetron or CP 93129 and paracetamol were evaluat-

ed separately. A two-way analysis of variance was used to analyse the effect of ondansetron or CP 93129 pre-treatment, paracetamol treatment and their interaction, followed by 2 × 2 factorial analysis by means of orthogonal comparisons. The level of significance was set at 0.05.

Results

Behavioural experiments

Paracetamol (400 mg/kg) significantly increased the %MPE values in the hot-plate test, as expected. At the dose of 4 mg/kg, ondansetron alone slightly but not significantly increased %MPE values (Fig. 1), while, at the doses of 2 and 4 mg/kg, it only slightly reduced the antinociceptive effect of paracetamol in a non-significant manner. ANOVA showed significance ($p = 0.001$) and the Student-Newman-Keuls test demonstrated that paracetamol- and ondansetron (2 and 4 mg/kg) plus paracetamol-values were significantly different from control values but not from each other. The interaction test showed no significance [$F(1–42) = 2.06$; $p > 0.05$ and $F(1–42) = 2.43$; $p > 0.05$ for ondansetron 2 and 4 mg/kg, respectively]. The effect of ondansetron was not dose-dependent as the increase in %MPE values provoked by paracetamol was reduced in a similar manner by the two doses (–17.1% and –18.5%, respectively).

Paracetamol (400 mg/kg) showed significant antinociceptive effect in the paw pressure test as well (Fig. 2). Ondansetron (at any dose used) did not affect the paw pressure test since the control values were no different from those of the treated rats and it did not antagonise the analgesic effect of paracetamol. ANOVA showed significance ($p = 0.002$) and the Student-Newman-Keuls test indicated that ondansetron (at any dose) plus paracetamol-values were no different from paracetamol ones; interaction test showed no significance [$F(1–42) = 0.04$; p 0.05 and $F(1–42) = 0.01$; $p > 0.05$ for ondansetron 2 and 4 mg/kg, respectively].

CP 93129, at the dose of 2 mg/kg, significantly prevented the analgesic effect of paracetamol in the hot-plate test (Fig. 3). ANOVA showed significance ($p = 0.001$): the Student-Newman-Keuls test indicated that CP 93129 (2 mg/kg) plus paracetamol-values were different from paracetamol ones; [interaction test: $F(1–56) = 4.24$; $p < 0.05$] while the lower doses were ineffective in inducing a antagonistic effect [$F(1–56) = 0.86$; $p > 0.05$ and $F(1–56) = 0.04$; $p > 0.05$ for 0.5 and 1 mg/kg, respectively].

CP 93129 (2 mg/kg) significantly antagonised the antinociceptive effect of paracetamol also in the paw pressure test (Fig. 4) [ANOVA: $p = 0.001$; interaction test: $F(1–56) = 8.7$; $p < 0.01$], while the lower doses were completely inactive since the values of CP 93129 (0.5 and 1 mg/kg) plus paracetamol were significantly higher than control values and similar to those of paracetamol alone [$F(1–56) = 0.08$; $p > 0.05$ and $F(1–56) = 0.01$; $p > 0.05$ for 0.5 and 1 mg/kg, respectively].

Receptor binding studies

In the three areas considered (frontal cortex, temporal-parietal cortex and midbrain) paracetamol was not able to modify either the maximum number (B_{max}) or the affinity constant (k_D) of 5-HT_3 receptors. Treatment with ondansetron (4 mg/kg i.p.) significantly increased the number of 5-HT_3 receptors in the three areas studied while ondansetron + paracetamol values were no different from those obtained with ondansetron alone (ANOVA: $p = 0.001$ for the three areas investigated). The affinity constant remained unaffected by any of the treatments (ANOVA: $p = 0.997$, $p = 0.131$ and $p = 0.991$ for frontal cortex, temporal-parietal cortex and midbrain, respectively) (Table 1).

Factorial analysis showed no interaction between ondansetron and paracetamol treatment in the frontal cortex [$F(1–28) = 1.31$, $p > 0.05$], the temporal-parietal cortex

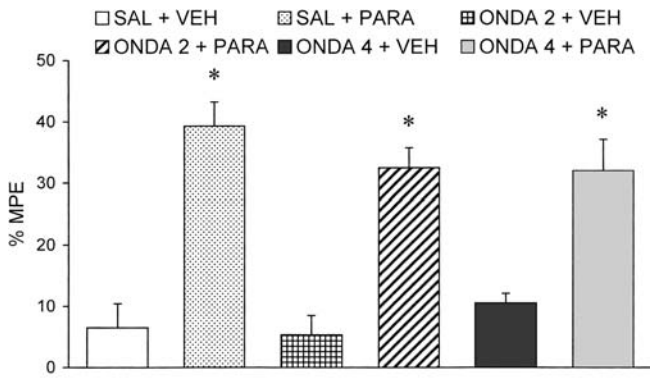

Fig. 1. Influence of ondansetron (ONDA, 2 or 4 mg/kg s.c.) pre-treatment on the antinociceptive action of paracetamol (PARA, 400 mg/kg i.p.) in the hot-plate test. Paracetamol was administered 15 min after ONDA and the rats were tested 30 min later. Each histogram represents the percentage of the maximum possible effect (%MPE); values were expressed as means ± SEM for eight rats for each group. SAL = saline VEH = vehicle consisting in 12,5% of 1,2 propanediol in sterile saline. *$p < 0.05$ vs control values (ANOVA followed by Student-Newman-Keuls test).

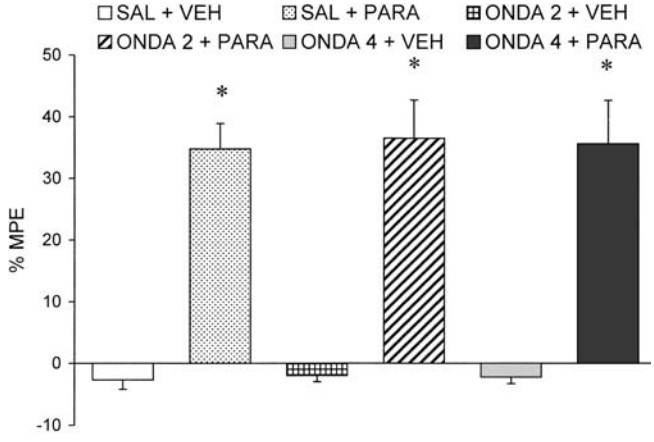

Fig. 2. Influence of ondansetron (ONDA, 2 or 4 mg/kg s.c.) pre-treatment on the antinociceptive action of paracetamol (PARA, 400 mg/kg i.p.) in the paw pressure test. PARA was administered 15 min after ONDA and the rats were tested 30 min later. Each histogram represents the percentage of the maximum possible effect (%MPE); values were expressed as means ± SEM for eight rats for each group. SAL = saline VEH = vehicle consisting in 12,5% of 1,2 propanediol in sterile saline. *$p < 0.05$ vs control values (ANOVA followed by Student-Newman-Keuls test).

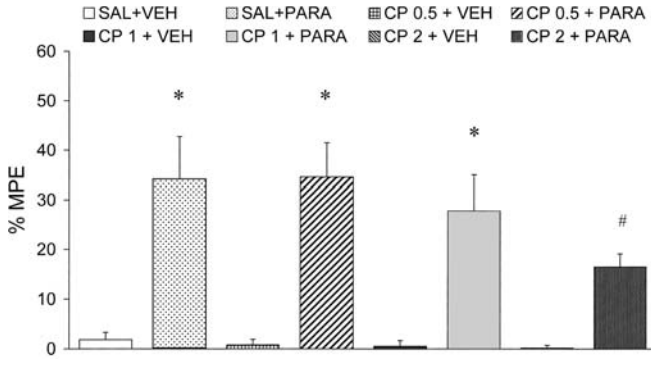

Fig. 3. Influence of CP 93129 (CP, 0,5, 1 or 2 mg/kg s.c.) pre-treatment on the antinociceptive action of paracetamol (PARA, 400 mg/kg i.p.) in the hot-plate test. PARA was administered 15 min after CP 93129 and the rats were tested 30 min later. Each histogram represents the percentage of the maximum possible effect (%MPE); values were expressed as means ± SEM for eight rats for each group. SAL = saline VEH = vehicle consisting in 12,5% of 1,2 propanediol in sterile saline. *p < 0.05 vs control values; *p < 0.05 vs SAL + PARA values (ANOVA followed by Student-Newman-Keuls test).

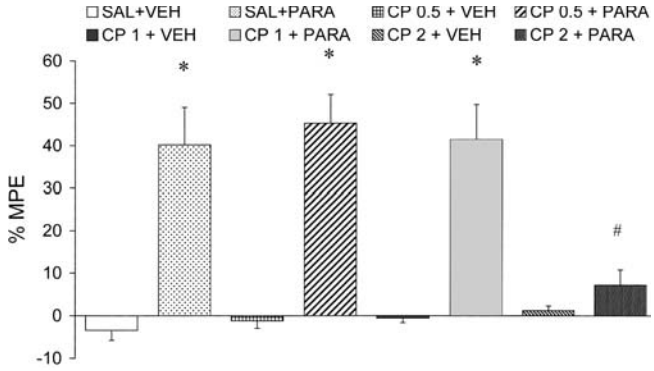

Fig. 4. Influence of CP 93129 (CP, 0,5, 1 or 2 mg/kg s.c.) pre-treatment on the antinociceptive action of paracetamol (PARA, 400 mg/kg i.p.) in the paw pressure test. PARA was administered 15 min after CP 93129 and the rats were tested 30 min later. Each histogram represents the percentage of the maximum possible effect (%MPE); values were expressed as means ± SEM for eight rats for each group. SAL = saline VEH = vehicle consisting in 12,5% of 1,2 propanediol in sterile saline. *p < 0.05 vs control values; *p < 0.05 vs SAL + PARA values (ANOVA followed by Student-Newman-Keuls test).

[F(1–28) = 3.47, p > 0.05] or the midbrain [F(1–28) = 0.12, p > 0.05].

Finally, paracetamol did not bind to 5-HT_3 binding sites. Competition experiments demonstrated that, at doses ranging between 10^{-9} and 10^{-4} M, paracetamol was not able to displace, in vitro, the binding of 1 nM [^{3}H]GR65630 in the three areas studied.

Discussion

The present results confirm that paracetamol (400 mg/kg) is able to induce an antinociceptive activity in the two algesimetric tests, the hot-plate test and the paw pressure test, in the rat, significantly increasing the %MPE values. The analgesic effect of paracetamol has been obtained after systemic administration in animals using a variety of behavioural tests chiefly in models devoid of inflammation, and similar results have been obtained in humans [22]. A spinal site of action has been suggested by many authors for the analgesic effect of paracetamol [4, 23, 24]. Moreover, the involvement of the central serotonergic system in the analgesia induced by non-opioid analgesics has been demonstrated [3], but the detailed mechanism by which serotonin acts, together with the exact nature of the receptor subtypes involved, has not yet been elucidated [25–27].

It has been suggested that serotonin modulates the response to noxious stimuli either at spinal or supraspinal levels: serotonin and serotonin agonists, injected both peripherally and in discrete brain areas, raise the nociceptive threshold, while serotonin receptor blockers attenuate analgesia at spinal and supraspinal levels [28, 29].

Some authors claim the involvement of spinal 5-HT_3 receptors in the antinociceptive effect of paracetamol [5, 9], suggesting an indirect action, since paracetamol does not bind to 5-HT_3 receptors. Our results confirm that paracetamol does not bind to 5-HT_3 receptors even at supraspinal level. On the other hand, our data demonstrate that, at supraspinal level, these receptors are not involved in the antinociceptive effect of paracetamol, in our conditions, as evaluated by means of the hot-plate and the paw pressure tests. Ondansetron alone increases the number of 5-HT_3 receptors; this effect was not modified by the treatment with paraceta-

Table 1. Influence of ondansetron on the effect of paracetamol on [^{3}H]GR65630 binding evaluations in the frontal and temporal-parietal cortex and midbrain of the rat.

Treatment	Frontal cortex		Temporal-parietal cortex		Midbrain	
	B_{max} (fmol/mg prot)	k_D (nM)	B_{max} (fmol/mg prot)	k_D (nM)	B_{max} (fmol/mg prot)	k_D (nM)
SAL + VEH	175.33 ± 20.22	1.68 ± 0.31	198.58 ± 14.41	1.61 ± 0.13	164.85 ± 11.30	1.26 ± 0.07
SAL + PARA	151.95 ± 8.42	1.62 ± 0.10	152.51 ± 9.54	1.41 ± 0.18	135.18 ± 9.68	1.28 ± 0.17
ONDA + VEH	291.27 ± 16.78*	1.87 ± 0.44	271.60 ± 21.42*	1.97 ± 0.24	265.02 ± 17.88*	1.33 ± 0.21
ONDA + PARA	308.71 ± 22.34*	1.73 ± 0.23	295.7 ± 23.23*	1.51 ± 0.12	244.20 ± 12.28*	1.31 ± 0.19

Ondansetron (ONDA, 4 mg/kg, s.c.) or saline (SAL) were administered 15 min before paracetamol (PARA, 400 mg/kg, i.p.) or vehicle (VEH, consisting in 12,5% of 1,2 propanediol in sterile saline) treatment. Rats were killed immediately after the hot-plate test (30 min after the last treatment) and the brain areas were weighed and frozen at –80 °C until assayed. Each value represents the mean ± SEM of 8 separate experiments and were derived from Rosenthal plot. B_{max} = maximum binding capacity: k_D = equilibrium dissociation constant. *p < 0.05 vs control values (ANOVA followed by Student-Newman-Keuls test).

mol, thus indicating that these two drugs do not display an interactive effect. In particular, paracetamol fails to affect the characteristics of 5-HT_3 receptors even in coadministration, as shown by the results of the interaction tests. This interesting effect of ondansetron on the number of 5-HT_3 receptors may be partially explained by the role of these receptors in the modulation of 5-HT release and by the involvement also of other neurotransmitters [30]. On the other hand, it is well known that the number of serotonin receptors is down- or up-regulated by the serotonin levels and by the interaction with 5-HT agonists and antagonists, respectively, after acute exposure and even in a relatively short period of time [31]. Indeed, the block of 5-HT_3 receptors by ondansetron may induce an up-regulation of these receptor subtype which allows paracetamol to exert its antinociceptive effect.

These results contribute to the body of conflicting evidence concerning the role of the 5-HT_3 receptor subtypes in the nociceptive activity of paracetamol [32]. The antinociceptive effect of paracetamol may be mediated by different serotonin receptor subtypes at spinal and supraspinal levels. This is suggested a) by the results obtained by some authors indicating that the activity of paracetamol is prevented by 5-HT_3 receptor antagonist tropisetron, when intrathecally (i.t.) injected [5] and b) by findings obtained in our laboratory showing that the antinociceptive effect of paracetamol, in the hot-plate test, is coupled to an increase in serotonin levels and a decrease in the number of 5-HT_2 receptors in the cerebral cortex and in the pons [6]. Thus, in some brain areas, the effect of paracetamol seems to be indirectly mediated through an increase in the serotonin levels regulated by 5-HT_2 receptors. As already mentioned, paracetamol does not act directly on serotonin receptors, as competition binding studies have demonstrated that it is not able to bind to 5-HT_{1A}, 5-HT_2 and 5-HT_3 receptors [33].

A possible explanation of the lack of antagonistic effect of ondansetron on the behavioural profile may be afforded by the heterogeneity observed between 5-HT_3 receptor antagonists, as suggested by Bardin and co-workers [34–35]: they show that the antagonism exerted by tropisetron and granisetron against the i.t. 5-HT antinociceptive effect differs in potency. Moreover, Courade and co-workers [27] demonstrate that tropisetron, but not granisetron inhibits the effect of acetaminophen in a mechanical pain test. On the other hand, in the present work we report that ondansetron per se does not induce any intrinsic effect in the two pain tests used, thus behaving like tropisetron in a mechanical pain test [35]. Indeed, tropisetron has been shown to be not very selective for 5-HT_3 receptors, as it binds also to other types of receptors (e.g. $GABA_A$). On the contrary, ondansetron and granisetron have little or no affinity for 5-HT_{1A} and 5-HT_2 receptors, α_1 and β_1 adrenoceptors, muscarinic, nicotinic, dopaminergic and H_1 or $GABA_A$ receptors in rat cerebral cortex and vagus nerve [36]. Therefore, the antagonistic effect on the analgesic activity of paracetamol induced by the less selective 5-HT_3 receptor antagonist tropisetron may be explained by its interaction with other types of receptor, while the more selective antagonists fail to produce any specific effect. The binding results obtained in the cortical areas and in the midbrain indicate that ondansetron produces the same effects in different areas involved in the serotonin-mediated pathways of the pain modulatory system. In fact, it has been suggested that the cortical areas are the end-point of the nociceptive system where the noxious information is perceived and processed [37, 38], while, in the midbrain, serotonin neurons, which project to the spinal cord and other brain regions (chiefly the limbic and cortical areas), are located.

It must also be borne in mind that these results have been obtained using different behavioural tests (mechanical and chemical pain) and routes of administration of drugs. The importance of the type of pain test employed has been underlined [39, 40]; in the hot-plate test, the antinociceptive effect is largely mediated by supraspinal mechanisms [41] and even the paw pressure test can involve supraspinal structures [42]. The lack of the antagonistic effect of ondansetron on the antinociception exerted by paracetamol in a thermal pain model is confirmed by using a mechanical pain model, the paw pressure test, thus suggesting that the negative results obtained by ondansetron treatment did not depend on the hot-plate test.

The diversity of effects exerted by paracetamol on nociception at spinal and supraspinal levels, reported in the literature, may be due to a different density of serotonin receptors subtypes [40]. Our results, however, are consistent with those obtained, at spinal level, by Courade and co-workers [27] using granisetron as 5-HT_3 antagonist, thus indicating that, also at supraspinal level, 5-HT_3 receptors do not seem to be involved in paracetamol-induced antinociception, as evaluated by means of two different pain models.

Moreover, we demonstrate that the 5-HT_{1B} receptor agonist CP 93129 can antagonise the effect of paracetamol in the two pain tests used. This result is in keeping with that of Roca-Vinardell and co-workers who showed an antagonist effect of CP93129 on the antinociceptive activity of paracetamol in the hot-plate test in mice [43]. CP 93129 is a specific 5-HT_{1B} receptor agonist, with 150 fold higher activity for 5-HT_{1B} versus other 5-HT_1, and 5-HT_2 binding sites [44]. It is also claimed to lack substantial affinity for dopamine, noradrenaline or opioid receptors [45]. The 5-HT_{1B} receptors present in the CNS of rodents seem to be autoreceptors associated with a decrease in transmitter release [46]; when stimulated, these receptors exert a negative feedback control of serotonin [17]. We suggest that this decrease in 5-HT levels exerted by CP 93129 may counteract the increase of serotonin levels induced by paracetamol preventing its antinociceptive effect.

These data add further evidence that serotonin is involved in the central effect of paracetamol via a complex net of receptor subtypes.

In conclusion, we suggest that the purported increase in 5-HT levels induced by paracetamol at a central level mediates an activation of 5-HT receptor subtypes, depending on the discrete brain areas investigated and on the region of the 5-HT inhibitory system taken into account. Finally, the role of 5-HT_{1B}, 5-HT_2 and 5-HT_3 receptors in the 5-HT-induced nociception still remains to be fully clarified as does the nature of the molecular mechanisms involved in the relationship involving paracetamol, serotonin and nociception.

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