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## Short Communication

# Effect of acute and repeated administration of paracetamol on opioidergic and serotonergic systems in rats

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**Abstract.** *Objective and design*: We investigated the antinociceptive effect of paracetamol or morphine after repeated administration and the changes in the characteristics of central μ-, κ- and 5-$HT_2$ receptors.
*Treatment:* Male rats were injected twice a day for seven days with paracetamol (400 mg/kg, i.p.) or morphine (5 mg/kg, s.c.).
*Methods:* The antinociceptive effect was evaluated 30 min after single and multiple doses of paracetamol and morphine through the hot-plate test. Binding techniques were used to evaluate the receptor characteristics in the frontal cortex.
*Results:* Both paracetamol and morphine induced an antinociceptive effect on day 1 but only paracetamol maintained this effect for seven days while morphine did not.
The number of μ-opioid receptors decreased on days 1, 3, and 7 by a similar percentage after paracetamol administration (by 29, 31 and 34 %, respectively), while morphine produced a progressive decrease in comparison with controls (by 37, 49 and 60 %, respectively) and κ-opioid receptors were unaffected. Both drugs similarly decreased the 5-$HT_2$ receptor number on all days of treatment (by about 30 %).
*Conclusions*: The opioidergic and serotonergic systems are involved in different ways in the induction and maintenance of antinociception after paracetamol or morphine treatment.

**Keywords:** Antinociception – Paracetamol – Morphine – Brain – μ-, κ- and 5-$HT_2$ receptors.

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## Introduction

The central mechanism of action of paracetamol is supported by many data both in humans and animals in different pain tests and after different routes of administration. The concentration in the central nervous system is dose dependent and comparable to that of its antinociceptive effects. Moreover, it has been demonstrated that paracetamol is able to cross the blood-brain barrier with a homogenous distribution in all brain areas [1].

The involvement of prostaglandins (PGs) in the mechanism of action of paracetamol has been proposed, taking into account the inhibition of the central cyclo-oxygenases (COX-1, COX-2 and COX-3) exerted by this drug [2–6], although the results are controversial in this regard. Moreover, interaction with many neurotransmitter systems, in particular with the serotonergic system, has been proposed to explain the effect of paracetamol: opioidergic, noradrenergic, serotonergic, cholinergic and NO-synthase systems have been studied in this regard [7, 8]. Indeed, paracetamol-induced antinociception seems to derive from the synergy between peripheral, spinal and supraspinal sites [9]. The supraspinal components identified in the mechanism of paracetamol are opioid-like and serotonergic [10, 11], the former contributing less than the latter [7], although the involvement of μ-opioid receptors in the antinociceptive action of paracetamol is still a matter of debate [12, 13]. We had previously detected an increase in serotonin concentration in the cerebral cortex and in the pons after acute paracetamol treatment. This effect is accompanied by a decrease in the central number of 5-$HT_2$ receptors [10].

It has been shown that the therapeutic activity of some non-opioid analgesic drugs can lead to problems of tolerance to their antinociceptive effects and it is well known that long-term administration of morphine produces tolerance to some behavioural effects, including analgesia in animals and hu-

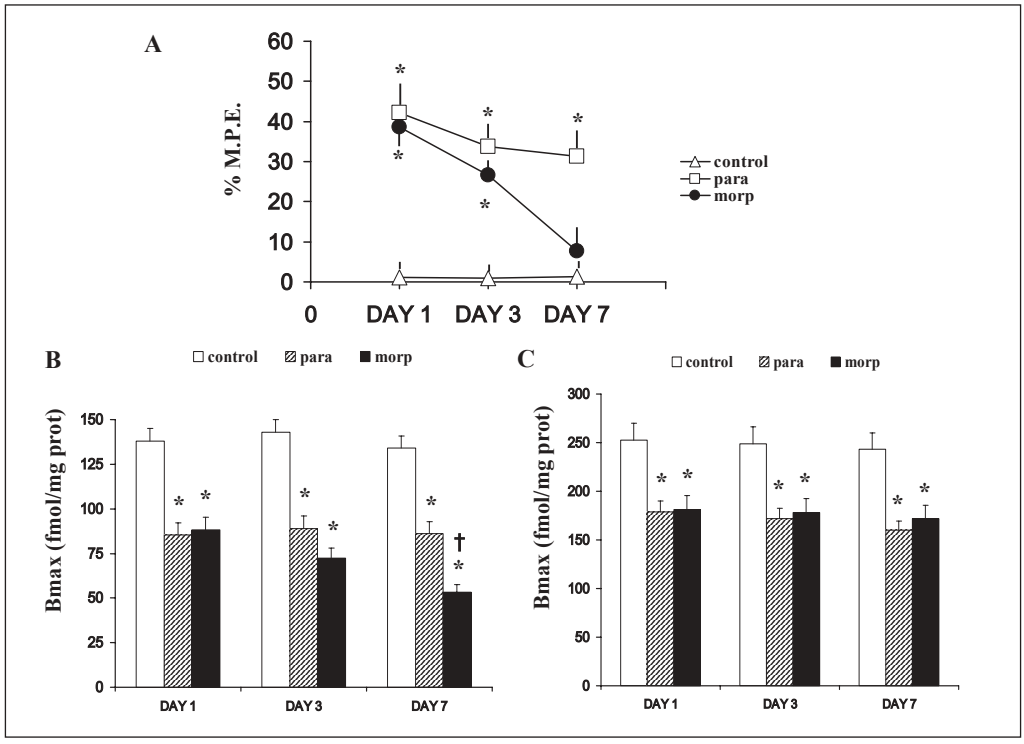


**Fig. 1. A)** The antinociceptive action of paracetamol (para: 400 mg/kg, i.p.) or morphine (morp: 5 mg/kg, s.c.) in the hot-plate test. Values are expressed as means ±SEM for 8 rats per group. **B)** [$^3$H]DAMGO and **C)** [$^3$H]ketanserin binding evaluations in the frontal cortex of the rat brain: effect of treatment with paracetamol or morphine thereon. Each value represents the mean ±SEM of 8 separate experiments. $B_{max}$ = maximum binding capacity.

*$p < 0.05$ vs. control. † $p < 0.05$ vs. para, same day. ANOVA followed by Student-Newman-Keuls test.

mans [14, 15]. The dose of paracetamol used in our present and previous works is high, when compared to its therapeutic use in humans [6], but its concentration remains below toxic levels in rats and mice [16, 17]. Indeed, it has been demonstrated that these high doses are essential in order to induce antinociceptive effects in rodents [18, 19].

A single treatment with paracetamol caused a rapid down-regulation of the 5-$HT_2$ receptors, while little or no evidence is present in the literature about the down-regulation of μ-receptors. Thus, the aims of the present work were to evaluate whether repeated administrations of paracetamol or morphine caused the phenomenon of tolerance to their antinociceptive effects in the hot-plate test and to assess possible changes in the characteristics of μ-, κ- and 5-$HT_2$ receptors in the frontal cortex of rats.

## Materials and methods

### *Animals*

Male Wistar rats, weighing 190–210 g at the beginning of the experiments, were housed in groups of two-three in controlled conditions. The ethical guidelines for the investigation of experimental pain in conscious animals were followed, and the procedures were carried out according to EC ethical regulations for animal research (EC Council 86/609; D.L. 27/01/1982, No. 116).

### *Drug treatment*

The rats were randomly divided into groups of eight animals. They were injected either with paracetamol (400 mg/kg i.p., dissolved in vehicle, which consisted of 12.5% of 1,2-propanediol in sterile saline) or vehicle, or with morphine (5 mg/kg s.c., dissolved in saline) or saline for seven consecutive days. The drugs were administered twice a day, at 8:00 and 18:00. The doses of paracetamol and morphine were chosen on the basis of previous dose-response experiments carried out under identical experimental conditions [10, 20]. The animals were subjected to the hot-plate test 30 min after the first drug administration on days 1, 3 and 7, each animal only being tested once. On days 1, 3 and 7, different groups of rats (N = 8 per group) were anaesthetised, decapitated and their brain removed immediately after pain threshold measurement. The frontal cortex was dissected and stored at –80 °C until required for analysis. Additional groups of rats were tested for motor activity 30 min after the same drug treatment on days 1, 3, and 7 (N = 8 per group).

### *Motor activity*

Motor activity was measured in an activity cage by means of ultrasound apparatus (Cibertec, S.A., Barcelona, Spain). The number of movements was recorded continuously between 8:00 and 11:00 a.m. in a soundproof room for 10 min, 30 min after drug injection on days 1, 3 and 7.

### *Hot-plate test*

The hot-plate consisted in 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.

### *Binding assays*

The characteristics of μ-receptors were evaluated according to the method of Hamon and co-workers (1987) [21] with minor modifications. Aliquots of membrane suspension were mixed with six concentrations

of [$^3$H]-D-Ala (2)-Me-Phe (4)-Gly-ol (5)-enkephalin ([$^3$H]DAMGO: 0.5–10 nM; specific activity, 57.5 Ci/mmol) and 1 µM naloxone for the determination of non-specific binding in a final volume of 0.5 ml.

The characteristics of κ-receptors were evaluated according to the method of Delvin and Shoemaker (1990) [22] with modifications. In saturation experiments, DAMGO (1 µM) and DSLET (5 µM) were added to each test tube to block µ- and δ-opioid sites. Membrane homogenates were added together with 0.25–8 nM [$^3$H]bremazocine (specific activity, 28.0 Ci/mmol) in a final volume of 0.5 ml and incubated at 37 °C for 45 min. Specific binding was determined with bremazocine (10 µM). Competition experiments used eight concentrations between 0.1 nM and 100 µM unlabelled paracetamol to displace 2 nM [$^3$H]bremazocine.

The characteristics of 5-HT$_2$ binding sites were evaluated according to the method of Leysen and co-workers (1982) [23] with minor modifications, as previously described by our group [10].

### *Statistical analysis*

The results of binding experiments were analysed according to the method of Rosenthal. The data were expressed as means ±SEM and correlated using analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. $p < 0.05$ was considered to be significant.

### *Drugs*

Paracetamol, morphine, methysergide, naloxone and bremazocine were purchased from Sigma Chemicals Co., Milan, Italy. [$^3$H]ketanserin, [$^3$H]DAMGO and [$^3$H]bremazocine were obtained from Du Pont NEN, Co. Ltd, Milan, Italy.

## Results

Acute treatment with either 400 mg/kg paracetamol or 5 mg/kg morphine provoked a significant and similar increase in the %MPE values. Both drugs maintained the effect also on day 3. On day 7, paracetamol had maintained its antinociceptive effect unchanged, while morphine had completely lost its effect [total ANOVA: $F(8\text{-}63) = 19.85$; $p < 0.001$] (Fig. 1A).

The motor activity of rats treated with paracetamol or morphine was compared with that of control rats on days 1, 3 and 7. No statistical difference in the total number of movements was observed between experimental and control groups [control values: 1382 ± 63, 1369 ± 70 and 1348 ± 78 on days 1, 3 and 7, respectively; total ANOVA $F(8\text{-}63) = 0.80$, $p = 0.608$].

In the frontal cortex, on day 1, paracetamol and morphine produced a significant and similar decrease in the µ-receptor number ($B_{max}$). After morphine treatment, the number of µ-receptors progressively decreased from day 3 to day 7. Paracetamol administration significantly decreased the number of µ-receptors at all times: the decrease was approximately the same on all study days [ANOVA: $F(8\text{-}63) = 22.23$, $p < 0.001$] (Fig. 1B). The affinity constants ($K_d$) remained unchanged after any treatment [control values: 0.92 ± 0.09, 1.07 ± 0.11 and 1.11 ± 0.14 on days 1, 3 and 7, respectively; ANOVA: $F(8\text{-}63) = 1.03$, $p = 0.476$].

The characteristics of κ-receptors were not affected by paracetamol or morphine at any time of evaluation. Moreover, paracetamol did not compete with [$^3$H]bremazocine binding sites (data not shown). The affinity constants did not change in any group [total ANOVA: $B_{max}$, $F(8\text{-}63) = 0.24$; $p = 0.982$; $K_d$, $F(8\text{-}63) = 0.23$, $p = 0.983$] (Table 1).

**Table 1.** Effect of single or repeated administrations of paracetamol or morphine on [$^3$H] bremazocine binding sites in the cerebral cortex of the rat.

| Treatment | Day 1 | Day 3 | Day 7 |
|---|---|---|---|
| Controls | $B_{max}$ 86.8 ± 7.7 | $B_{max}$ 71.9 ± 5.6 | $B_{max}$ 84.7 ± 10.1 |
| | $K_d$ 1.69 ± 0.51 | $K_d$ 1.72 ± 0.33 | $K_d$ 1.78 ± 0.21 |
| Paracetamol | $B_{max}$ 82.4 ± 14.3 | $B_{max}$ 77.8 ± 10.4 | $B_{max}$ 73.9 ± 19.4 |
| 400 mg/kg | Kd 1.87 ± 0.21 | Kd 1.92 ± 0.31 | Kd 1.80 ± 0.21 |
| Morphine | $B_{max}$ 78.4 ± 7.2 | $B_{max}$ 69.4 ± 5.7 | $B_{max}$ 77.3 ± 17.3 |
| 5 mg/kg | $K_d$ 2.01 ± 0.12 | $K_d$ 1.64 ± 0.32 | $K_d$ 2.03 ± 0.13 |

Values are means ± SEM of 8 separate experiments. $B_{max}$ (fmol/mg prot.) = maximum binding capacity $K_d$ (nM) = equilibrium dissociation constant. ANOVA did not reach significance for either parameter.

As shown in Figure 1C, after acute or repeated administrations paracetamol and morphine similarly decreased the number of 5-HT$_2$ binding sites, the effect being of the same order of magnitude on days 1, 3, and 7 [ANOVA: $F(8\text{-}63) = 15.04$; $p < 0.001$]. The affinity constants ($K_d$) remained unchanged after any treatment [control values: 1.34 ± 0.18, 1.48 ± 0.07 and 1.28 ± 0.16 on days 1, 3 and 7, respectively; ANOVA: $F(8\text{-}63) = 0.66$, $p = 0.728$].

## Discussion

Our data indicate a difference in the effect of paracetamol and morphine on non-inflammatory pain after repeated administrations, since morphine lost its analgesic effect while paracetamol maintained it, as also described by other authors [24].

In this experimental model both morphine and paracetamol exerted their analgesic effect without affecting motor activity.

Paracetamol decreased the number of µ-receptors in a similar way at all evaluation times, while morphine produced a time-dependent decrease: this difference may occur as a consequence of the low paracetamol affinity for µ-receptors [11] and may partially account for the lack of tolerance in the antinociceptive or biochemical effects exerted by this drug after 7 days of treatment.

Neither paracetamol nor morphine modified the characteristics of κ-receptors, suggesting that tolerance to the analgesic effect of morphine does not depend on these receptors.

Moreover, the 5-HT pathways seem to have little bearing on the phenomenon of tolerance, since the 5-HT$_2$ receptor change follows the same pattern after repeated administrations with both drugs, while it may be involved in their antinociceptive activity. Indeed, several studies demonstrate that systemic morphine exerts its analgesic effect, at least in part, through the serotonergic system, increasing the release of serotonin in some brain areas [25] and in synaptosomes of the rat cerebral cortex, as do also other µ-opioid agonists [26].

The opioidergic and serotonergic pathways are closely interconnected and can interact to modulate and produce

many behavioral changes, including nociception (see Millan, 2002, for a review) [27].

The similar efficacy found in the acute antinociceptive effect of these two compounds at the chosen doses under our experimental conditions, despite belonging to different classes of drugs, may be explained by the fact that both can affect either the serotonergic or the opioidergic systems, although at various degrees. On the other hand, the differential influence of morphine and paracetamol on other transmitters/modulators involved in nociception could explain the diverse development of tolerance displayed by these two drugs. The involvement of supraspinal neurotransmitter systems, accompanying this phenomenon, does not exclude other mechanisms, including the interaction between PG metabolism and the serotonergic system [4], or the implication of the central cannabinoid system in the antinociceptive effect of paracetamol [28].

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