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The dose-related effects of paracetamol on hyperalgesia and nociception in the rat

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1 We have studied the effects of 3 low doses of paracetamol (25, 50 and 100 mg kg^{-1} p.o.) on inflammatory hyperalgesia, inflammatory oedema, and nociceptive thresholds in rats.

2 At the lower dose (25 mg kg^{-1}), paracetamol reduces only central hyperalgesia.

3 At the doses of 50 and 100 mg kg^{-1}, paracetamol reduces also peripheral hyperalgesia; moreover, it enhances nociceptive thresholds to a mechanical stimulus in the non-inflamed paws.

4 Neither paw inflammatory oedema nor tail nociceptive thresholds to a thermal stimulus were modified by paracetamol administration.

5 Our results suggest that paracetamol can reduce hyperalgesia without affecting physiological nociception and inflammation.

Keywords: Inflammation; hyperalgesia; nociception; paracetamol

Introduction

For many years, it has been accepted that the analgesic effect of non-steroidal anti-inflammatory drugs (NSAIDs) was due to the reduction of inflammatory processes by the inhibition of prostaglandin synthesis. However, there is now evidence to suggest that inhibition of locally synthesized prostaglandins does not completely explain the analgesic effect of these drugs in a number of experimental and clinical models of pain (McCormack, 1994; McCormack & Urquhart, 1995). In accordance with these observations, it is well known that paracetamol, a drug widely prescribed for the treatment of mild to moderate pain, has weak anti-inflammatory actions and exerts its analgesic effect without inducing a noticeable prostaglandin-synthetase inhibition in peripheral damaged tissues (Clissold, 1986). Nevertheless, the mechanism of its analgesic action is still not totally clear. It has been suggested that the antinociceptive action of paracetamol is centrally mediated (Carlsson & Jurna, 1987; Tjolsen *et al.*, 1991). In recent years it has been demonstrated that central sensitization in the spinal cord plays a fundamental role in pain perception (Woolf, 1994), and that paracetamol exerts a direct spinal action by blocking the hyperalgesia induced by the activation of spinal glutamate and substance P receptors (Hunskaar *et al.*, 1985; Bjorkman *et al.*, 1994). Furthermore the results of clinical investigations would appear to indicate that paracetamol exerts a direct action within the central nervous system (Piletta *et al.*, 1991). The experimental models usually used for the study of analgesic drugs in animals enable us to evaluate their ability to enhance nociceptive thresholds. However, it is well known that, in contrast to nociceptive pain, clinical pain does not represent a protective response to noxious stimuli, but it is characterized by the appearance of abnormal hypersensitivity, a substantial component of which is generated within the central nervous system (Woolf, 1994). For this reason, experimental models of hyperalgesia seem to be more reliable as an indication of the clinical efficacy of the analgesic drugs.

It has been shown that, at high doses, paracetamol also reduces experimental inflammation (Glenn *et al.*, 1977). In this study we have evaluated the effects of low doses of paracetamol on two different models of inflammatory hyperalgesia in rats.

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Methods

Sprague-Dawley CD male rats (Charles River, Calco, Italy), eight in each experimental group, were used in all experiments. The animals, 200–250 g body weight, were housed at 22°C with a light : dark cycle of 14 : 10 h, with food and water *ad libitum*. All experiments began between 09 h 00 min–09 h 30 min.

The inflammatory agent (10% brewer's yeast suspension) was injected subcutaneously in the plantar part of the left hindpaw, in a volume of 0.1 ml. The control animals were injected with a volume of saline (0.9% NaCl) equal to that of irritant substance.

Paracetamol was dissolved in 0.25% methocel in 0.9% NaCl and administered orally at doses of 25, 50 and 100 mg kg^{-1}, 1 h before yeast injection. The control animals received an equal volume of vehicle.

The inflammatory oedema was evaluated by the measurement of the hindpaw swelling induced by the injection of yeast suspension or saline (Bianchi *et al.*, 1994b). We used a 7150 Plethysmometer (Basile, Comerio, Italy); the hindpaw was submerged to the tibiotarsal joint into the water filled cell of the instrument. The volume of displacement, which is equal to the paw volume, was then read on a digital display. The results are expressed as the algebraic difference between the volume of the treated and untreated hindpaw.

The Randall-Selitto paw-withdrawal test (Randall *et al.*, 1957) was used to measure mechanical nociceptive thresholds and peripheral, inflammatory hyperalgesia. The stimulus was applied with an analgesymeter (Basile, Comerio, Italy) which generates a linearly increasing mechanical force, applied by a conical piece of plastic with a dome-shaped tip on the dorsal surface of the rat's hindpaw. The results represent the maximal pressure (expressed in g) tolerated by the animal. To avoid tissue damage, only a trial was performed at each time point.

The tail-flick test was used to assess the nociceptive thresholds in the normal rats and the central hyperalgesia in the animals with the inflamed hindpaw (Bianchi *et al.*, 1994a). The noxious stimulus consisted of a radiant heat from a lamp focused onto a 2.0×2.0 mm area of the dorsal surface of the rat's tail. The stimulus was applied 3 cm from the distal end of the tail. The timer of the tail-flick apparatus, precise to 0.1 s, was stopped by a photocell when a tail-flick occurred. Basal values were in the range of 3.0–4.0 s and the cut-off time was 8 s. Treatments were administered only to the rats in which basal tail-flick latency had remained stable for three sub-

sequent measurements. Afterwards, in order to prevent tissue damage, only one tail-flick response was measured per time point. Hyperalgesic state was assessed by delta reaction time (basal latency − test latency). Statistical analysis was performed by Analysis of Variance (ANOVA), followed by Tukey's test for multiple comparisons.

Results

In conformity with our previous data, the yeast solution injection in the left hindpaw induced a significant reduction of tail flick latencies (Figure 1). This figure also shows that the central hyperalgesia had already been completely abolished by pretreatment with the lower dose of paracetamol (25 mg kg^{-1} p.o.). Therefore, the effect of the administration of the higher doses (50 and 100 mg kg^{-1}) on the central hyperalgesia was apparently identical to that produced by the lower dose (data not shown).

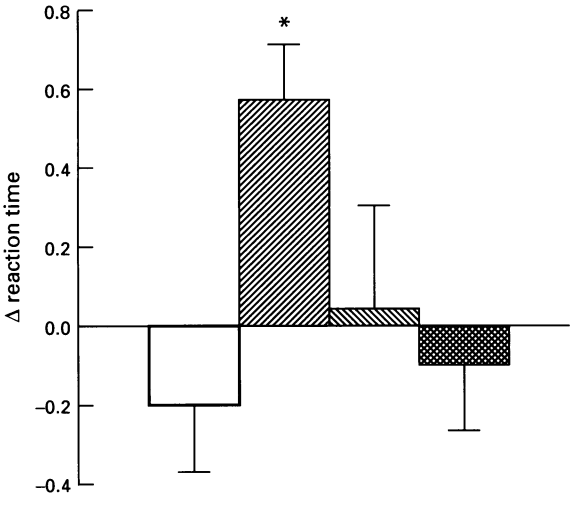

Figure 1 Effect of paracetamol (25 mg kg^{-1} p.o.) on the reduction of tail withdrawal response (central hyperalgesia) induced by peripheral inflammation. The evaluation was performed by tail-flick test, 4 h after yeast administration. Values are means ± s.e.mean of the algebraic difference between basal and test latencies (delta reaction time). (□) Controls; (▨) saline + yeast; (▩) paracetamol + yeast; (▩) paracetamol + saline. *$P < 0.005$ vs controls vs paracetamol + yeast, and vs paracetamol + saline.

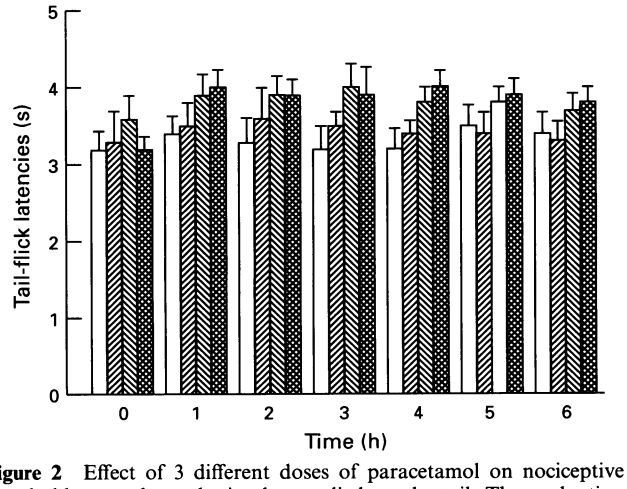

Figure 2 Effect of 3 different doses of paracetamol on nociceptive thresholds to a thermal stimulus applied on the tail. The evaluation was performed by the tail-flick test. (□) Controls; (▨) paracetamol 25 mg kg^{-1}; (▩) paracetamol 50 mg kg^{-1}; (▩) paracetamol 100 mg kg^{-1}. Values are means ± s.e.mean.

We have indicated only the results of the observations performed 4 h after yeast administration, since we have observed that, at this point in time, the inflammatory process and the hyperalgesic condition were more evident (Bianchi *et al.*, 1994a, b, c). It is interesting to note that a dose of 25 mg kg^{-1} of the drug did not have any effect on the rats without peripheral inflammation (Figure 2). This figure also shows that higher doses of paracetamol did not affect nociceptive thresholds in the tail-flick test. Moreover, neither paw oedema nor paw hyperalgesia were affected by the administration of paracetamol 25 mg kg^{-1} (Table 1 and Figure 3a). At both the doses of 50 and 100 mg kg^{-1}, paracetamol reduced paw hy-

Table 1 Effect of paracetamol on paw oedema induced by an injection of brewer's yeast

Treatment	*Δ* (ml)
Vehicle	1.96 ± 0.90
Paracetamol 25 mg kg^{-1}	1.85 ± 0.13
Paracetamol 50 mg kg^{-1}	1.82 ± 0.12
Paracetamol 100 mg kg^{-1}	1.91 ± 0.60

Values are means ± s.e.mean of the algebraic difference between the volume of treated and untreated paws. The evaluation was performed by plethysmometry, 4 h after yeast administration.

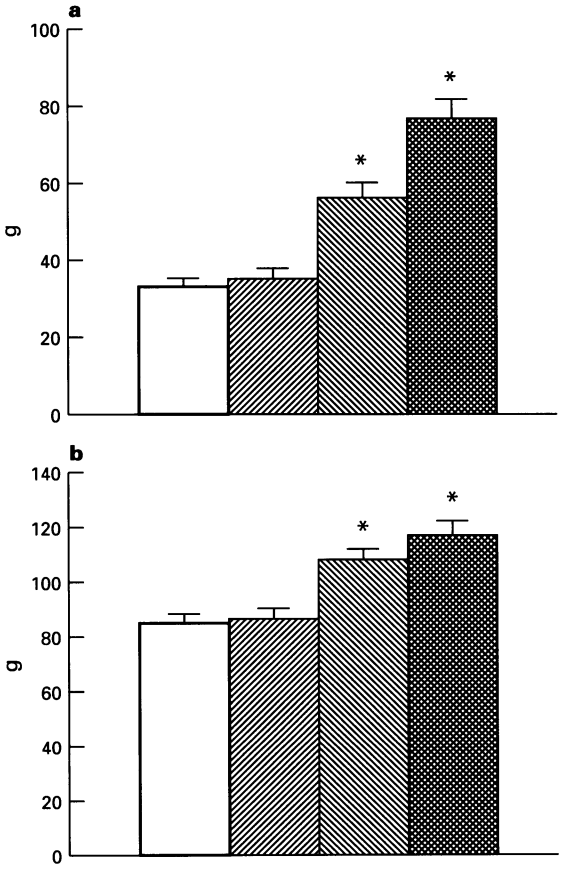

Figure 3 (a) Effect of 3 different doses of paracetamol on paw hyperalgesia induced by an injection of brewer's yeast. *$P < 0.001$ vs vehicle + yeast. (b) Effect of 3 different doses of paracetamol on nociceptive thresholds to a mechanical stimulus applied to the non-inflamed paws. *$P < 0.001$ vs controls. The evaluations were performed by Randall-Selitto test, 4 h after yeast administration. (□) Controls; paracetamol, (▨) 25 mg kg^{-1}, (▩) 50 mg kg^{-1}, (▩) 100 mg kg^{-1}. Values are mean ± s.e.mean.

peralgesia (Figure 3a), but not paw oedema (Table 1). After the administration of the two higher doses, we observed a significant enhancement of nociceptive thresholds to a mechanical stimulus also in the non-inflamed paws (Figure 3b).

Discussion

We have previously shown that using the tail-flick test it is possible to demonstrate a central facilitation of the responses to a noxious peripheral stimulus, probably due to the overlapping fields of afferent fibres at the spinal level (Bianchi *et al.*, 1994a). In this study, we demonstrate that a low dose of paracetamol is able to prevent such a kind of hyperalgesia. Curiously, this dose of the drug does not reduce peripheral hyperalgesia, nor inflammatory oedema. Moreover, neither the nociceptive thresholds to thermal or mechanical stimuli were modified by the administration of paracetamol 25 mg kg^{-1}. Therefore, our data support the hypothesis that paracetamol can reduce hypersensitivity at a central level.

At the doses tested, paracetamol does not affect the development of inflammatory oedema. Thus, the observations made after the administration of two higher doses of the drug confirm the dissociation between the anti-inflammatory and the antinociceptive action of paracetamol (McCormack & Brune, 1991).

Our experiments do not put us in a position to speculate on the possible mechanism involved in the anti-hyperalgesic action of paracetamol. However, the experimental model that we used indicates that the drug is able to affect central modulation of nociceptive inputs. It has been observed that spinal cyclooxygenase is involved in the modulation of nociceptive inputs (Malmberg & Yaksh, 1992; McCormack, 1994) and that prostaglandins affect descending inhibitory systems by reducing the release of monoamines at the spinal level (Taiwo & Levine, 1988). It has also been observed that paracetamol is an inhibitor of prostaglandin synthesis in the central nervous system (Clissold, 1986) and in glia cells (Lantz *et al.*, 1986). Therefore, we cannot exclude an involvement of spinal cyclooxygenase in the anti-hyperalgesic effect of this drug.

It is well known that several biochemical changes emerge in the experimental conditions of hyperalgesia. In particular, it has been demonstrated that N-methyl-D-aspartate (NMDA) produces a facilitation of the nociceptive tail-flick reflex (Meller *et al.*, 1992) and that the activation of spinal NMDA receptors is involved in the hypersensitivity that follows peripheral tissue inflammation (Ren *et al.*, 1992). Consistent with this observation, we have previously shown that a competitive antagonist of NMDA receptors blocks the central hyperalgesic condition induced by the yeast injection in the hindpaw (Bianchi *et al.*, 1994a). As it has been observed that paracetamol is able to antagonize the spinal hyperalgesia induced by NMDA (Björkman *et al.*, 1994), the involvement of spinal excitatory aminoacids in the anti-hyperalgesic action of paracetamol should be considered. Moreover, it has been demonstrated that, after peripheral administration, paracetamol reduced pain-related behaviour induced by intrathecally administered substance P (Hunskaar *et al.*, 1985). It has been recently argued that the inhibition of L-arginine-nitric oxide pathway might represent a central mechanism of action for paracetamol (Björkman *et al.*, 1994). All these results fit in well with our behavioural observations indicating that paracetamol can modulate the neuronal processes involved in the processing of nociceptive inputs at the spinal level and may explain our findings.

Hopes have been expressed that drugs will be developed that act specifically on central sensitization (Woolf, 1994). Until such novel, anti-hyperalgesic drugs have been produced, we think that paracetamol deserves more attention as an anti-hypersensitivity agent also for use in clinical conditions.

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