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Paracetamol exerts a spinal, tropisetron-reversible, antinociceptive effect in an inflammatory pain model in rats

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Abstract

Experiments were performed in carrageenin-treated rats to study, the antinociceptive and anti-inflammatory effects of paracetamol intravenously (i.v.) or intrathecally (i.t.) injected on rats submitted to a mechanical noxious stimulus. The influence of intrathecal tropisetron, a 5 hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonist, on the antinociceptive effects of paracetamol, was also studied. Paracetamol induced a significant antinociceptive effect after (100, 200 and 300 mg/kg) i.v. and (50, 100 and 200 μg/rat) i.t. injection, but no change occurred on edema volume. The effect of paracetamol was totally inhibited by tropisetron (10 μg/rat, i.t.). The foregoing results demonstrate that, in conditions of inflammatory pain, paracetamol exerts a central antinociceptive effect involving spinal 5-HT$_3$ receptors, without inducing any anti-inflammatory action. These data, give further arguments to consider paracetamol as a central analgesic drug which must be distinguished from non-steroidal anti-inflammatory drugs (NSAIDs), which justifies the usual combination of paracetamol in post-operative pain.

Keywords: Paracetamol; Tropisetron; 5-HT$_3$ receptor; Spinal cord; Inflammation; (Rat)

1. Introduction

Paracetamol is often classified in the group of aspirin-like (Ferreira, 1972) or non-steroidal anti-inflammatory-like (Clissold, 1986; Insel, 1996) drugs. However, it does not share the same profile both in terms of therapeutic activities and side effects, considered, for non-steroidal anti-inflammatory drugs (NSAIDs), to be due, at least in part, to the inhibition of the synthesis of prostaglandins (Insel, 1996). These marked differences suggest that their mechanisms of action may differ. In vitro, paracetamol weakly inhibits cyclooxygenases, compared to several NSAIDs (Mitchell et al., 1994). Clinical experiments have shown that therapeutic doses of paracetamol failed to reduce 6-keto-prostaglandins $F_{1\alpha}$ urinary excretion (Seppälä et al., 1983) or prostaglandins E_2 synovial fluid levels (Bippi and Fröhich, 1990). Finally, Vane (1971) demonstrated that paracetamol weakly inhibits peripheral cyclo-oxygenases but has a more potent effect on the centrally located enzymes. This limited inhibition of cyclooxygenases, especially of peripheral cyclooxygenases, led several authors to propose a central mechanism of action of paracetamol (Carlsson et al., 1988; Piletta et al., 1991; Bannwarth et al., 1995).

Such a hypothesis is in line with the ability of paracetamol to cross the blood–brain barrier both in rats (Courade et al., 2001b) and humans (Bannwarth et al., 1992), and with its efficacy both in animal pain models after central administration (Alloui et al., 1996; Pelissier et al., 1996) and in models devoid of any inflammation and only sensitive to centrally acting drugs (Carlsson and Jurna, 1987). Some neurobiochemical hypotheses have been proposed for this centrally mediated effect since paracetamol reduces behavior induced by intrathecally injected substance P or *N*-methyl-D-aspartate (NMDA) (Björkmann et al., 1994); besides, several authors have demonstrated a serotonergic involvement in the antinociceptive effect of paracetamol (Tjolsen et al., 1991; Pini et al., 1996; Alloui et al., 1996; Pelissier et al., 1996; Srikiatkhachorn et al., 1999; Sandrini et al., 1999). However, all these studies have been

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performed on healthy animals subjected to acute noxious stimulation, which is not optimal in terms of clinical predictability.

Given this limitation and divergent opinions about the anti-inflammatory effect of paracetamol (Honoré et al., 1995; Wong and Gardocki, 1983), we have performed a work in a model of maintained inflammatory pain. The aims of this work are: (1) to determine comparatively the anti-nociceptive and anti-inflammatory effects of paracetamol, (2) to look for the existence of a central effect in an inflammatory pain model, and (3) to determine the influence of i.t. tropisetron, a 5-HT_3 receptor antagonist, in the same model. If a central, 5-HT_3 receptor-dependent mechanism exists in conditions of inflammatory pain, this would give a basic justification for the ongoing practice of clinically combining paracetamol and NSAIDs.

2. Materiel and methods

2.1. Animals

Male Sprague–Dawley rats (200–250 g) (Charles River, St-Aubin-Lès-Elbeuf, France) were used. After their arrival at the laboratory, animals were allowed to acclimatize for 1 week in groups of six rats per cage with free access to food and water.

2.2. Induction of inflammation and nociceptive test procedures

Inflammation was induced by the subcutaneous injection of 0.2 ml 2% solution of the polysaccharide carrageenin in saline (0.9% NaCl) into the plantar right hind paw. The edema volume of the right hindpaw was measured by plethysmometer (Apelex 7150, Massy France). The anti-nociceptive effect of the tested compounds was assessed using mechanical noxious stimulation as previously described by Randall and Selitto (1957). Nociceptive thresholds, expressed in grams, were measured with a Ugo Basil analgesimeter (Apelex Massy France, tip diameter of the probe: 1 mm) by applying increasing pressure to both hind paws until a squeak (vocalization threshold) is obtained. Since a certain amount of suffering might result from these experiments, the guidelines of the Committee for Research and Ethical Issue of the IASP (Zimmerman, 1983) were followed and approved by the local Ethics committee. Great care was taken, particularly with regard to housing conditions, to avoid or minimise discomfort of the animals.

2.3. Experimental protocol

The experiments were performed "blind" in a quiet room by a single experimenter. For each experimental series, the method of blocks was used to allow assessment of the effect of different randomised treatments in the same lapse of time, and to avoid any uncontrolled experimental influence. Different animals were used for each experiment.

2.3.1. Effect of systemic administration of paracetamol

Before injection of carrageenin, two stable vocalisation threshold values from the two hind paws were determined (220.4 ± 5.1 and 217.4 ± 3.1 g, for right and left hind paws, respectively, $n = 24$). Two hours after carrageenin injection, vocalisation thresholds were decreased (157.3 ± 5.0 and 174.1 ± 4.6 g, respectively, just before the injection of paracetamol, $n = 24$). Paracetamol (100, 200 and 300 mg/kg, $n = 6$ per dose) or vehicle (1 ml/kg, $n = 6$) was then intravenously injected. Vocalization thresholds were determined 15, 30, 45, 60, 75, 90, 105 and 120 min after paracetamol administration. The edema volume was assessed with an Apelex 7150 plethysmometer before injection of carrageenin, 2 h after that and then every 30 min until 120 min after injection of paracetamol or vehicle.

2.3.2. Effect of intrathecal injection of paracetamol

Before injection of carrageenin, two stable vocalisation threshold values were determined (210 ± 3.9 and 206.5 ± 4.0 g for right and left hind paws, respectively, $n = 24$). These thresholds decreased to 160.4 ± 4.6 and 177.5 ± 5.1 g, respectively ($n = 24$), 2 h after injection of carrageenin. Paracetamol (50, 100 and 200 μg/rat, $n = 6$ per group) and saline (10 μl/rat, $n = 6$) were then intrathecally (i.t.) injected according to the method described by Mestre et al. (1994). Before injection, rats were slightly anaesthetized with volatile isoflurane (3.5%). The vocalization thresholds of rats were determined 15, 30, 45, 60, 75, 90, 105 and 120 min after paracetamol administration. Edema volume was assessed before injection of carrageenin, 2 h after and then every 30 min until 120 min after injection of paracetamol or saline.

2.3.3. Influence of tropisetron on the antinociceptive effect of paracetamol

Two experiments were performed to assess the influence of tropisetron according to the injection time of paracetamol before or after carrageenin. These two experiments were justified by previous results showing that the effect of NSAIDs on this model can change according to their time of injection, before or after carrageenin (Randall and Selitto, 1957; Winter and Flataker, 1965) These differences could be due to the kinetics of the release of peripheral mediators involved in inflammation (Guilbaud et al., 1986). If paracetamol exerts a tropisetron-reversible central effect, its effect and the influence on the antagonist would be similar in the two experimental conditions.

2.3.3.1. Post-injury administration of paracetamol. Two hours after the injection of carrageenin, two stable threshold values from the two hind paws were determined (139.7 ± 6.7 and 169.7 ± 5.4 g for right and left hind paws, respectively, $n = 24$). They illustrated a mechanical hyperalgesia (pre-carrageenin vocalisation thresholds values: 178.2 ± 4.7

and 188.6 ± 4.1 g, respectively, $n = 24$). Tropisetron (10 μg/rat) or saline (10 μl/rat) were intrathecally injected (under slight anaesthesia with volatile isoflurane) then, paracetamol (200 mg/kg) or vehicle (1 ml/kg) being intravenously administered 5 min after. Pain scores were determined 15, 30, 45, 60, 75, 90, 105 and 120 min thereafter. The edema volume was assessed before any treatment, 2 h after injection of carrageenin and then every 30 min until 120 min after injection of paracetamol or vehicle. Four groups were studied: saline + vehicle, tropisetron + vehicle, saline + paracetamol and tropisetron + paracetamol ($n = 6$ for each group).

2.3.3.2. Pre-injury administration of paracetamol. Two stable vocalisation threshold values from the two hind paws were determined before any treatment (210.4 ± 3.7 and 197.1 ± 4.6 g, for right and left hind paws, respectively, $n = 24$). Just after i.t. injection of either tropisetron (10 μg/rat) or saline (10 μl/rat) was performed (under slight anaesthesia with volatile isoflurane), followed 5 min after by administration of paracetamol (200 mg/kg, i.v.) or vehicle (1 ml/kg, i.v.). Injection of carrageenin into the right hind paw was performed 5 min after injection of paracetamol or vehicle. Pain scores were determined 15, 30, 45, 60, 75, 90, 105 and 120 min after injection of carrageenin. The edema volume was assessed before any treatment, and then every 30 min until 120 min after injection of carrageenin. Four groups were studied: saline + vehicle, tropisetron + vehicle, saline + paracetamol and tropisetron + paracetamol($n = 6$ for each group).

2.4. Drugs

Because paracetamol is weakly soluble in saline, its prodrug propacetamol, Prodafalgan® (UPSA/BMS Laboratories, Rueil-Malmaison, 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 paracetamol in the corresponding experiments; the doses expressed correspond to doses of paracetamol. Vehicle was the solvent (trisodic citrate, 0.02 g/ml) of propacetamol. For i.t. injection, paracetamol (and not propacetamol) (UPSA/BMS, Laboratories) and tropisetron (Sandoz, Paris, France) were dissolved in saline (10 μl/rat).

2.5. Statistics

Results were expressed in grams (g) as mean ± S.E.M. To assess overall effect, areas under the time-course curves (AUC) of the antinociceptive effects were calculated using the trapezoidal method. The values used for calculating AUC was the individual variations in vocalisation thresholds (postdrug values – predrug values) to avoid any influence of the individual variation in the control predrug values. Data were analyzed by two-way analysis of variance (ANOVA) followed by the predicted least statistical differences (PLSD) Fischer *t*-test when the time-course of the effect was studied. A one-way analysis of variance was used to compare the effect of different treatments as estimated by AUC. The significance level was $P < 0.05$ for the two statistical analyses.

3. Results

3.1. Antinociceptive effect of systemic administration of paracetamol

Paracetamol, when systemically injected, increased significantly the vocalization thresholds. The greatest increases in pain thresholds obtained with doses of 100, 200 and 300 mg/kg were +130.0 ± 18.8 g (+84.3 ±14.5%), +138.3 ± 24.6 g (+90.7 ± 16.3%) and +196.6 ±

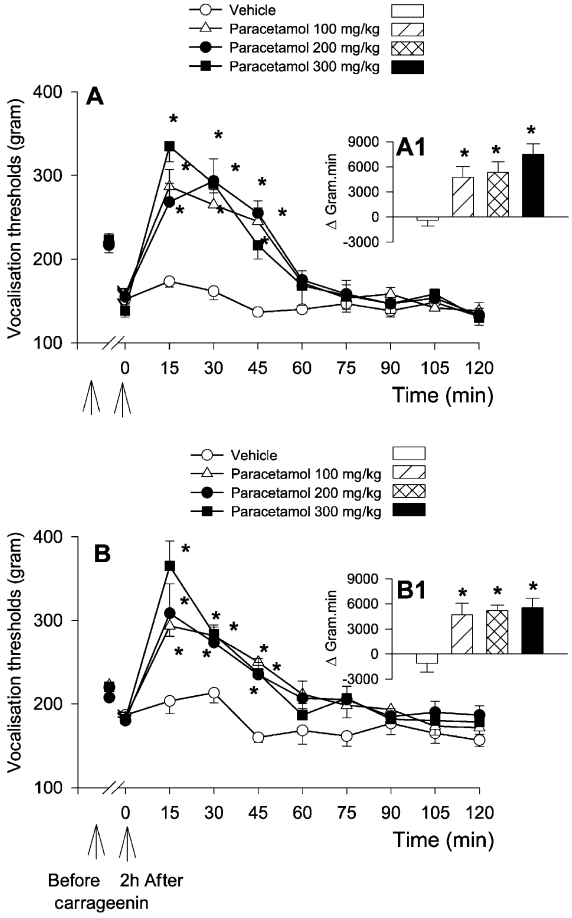

Fig. 1. Effect of intravenous administration of paracetamol on the vocalization thresholds to paw pressure in carrageenin-treated rats. Results are expressed (A, B) by the time-course curve of mean ± S.E.M. vocalisation thresholds (g) and (A1, B1) by the mean ± S.E.M. area under the time-course curves (AUC) of the variations (postdrug – predrug values of vocalisation thresholds), calculated by the trapezoidal method. Rats were treated with either vehicle or paracetamol (100, 200 and 300 mg/kg, i.v.) ($n = 6$ per group) administrated 120 min after intraplantar injection of carrageenin. * $P < 0.05$ vs. vehicle-treated group.

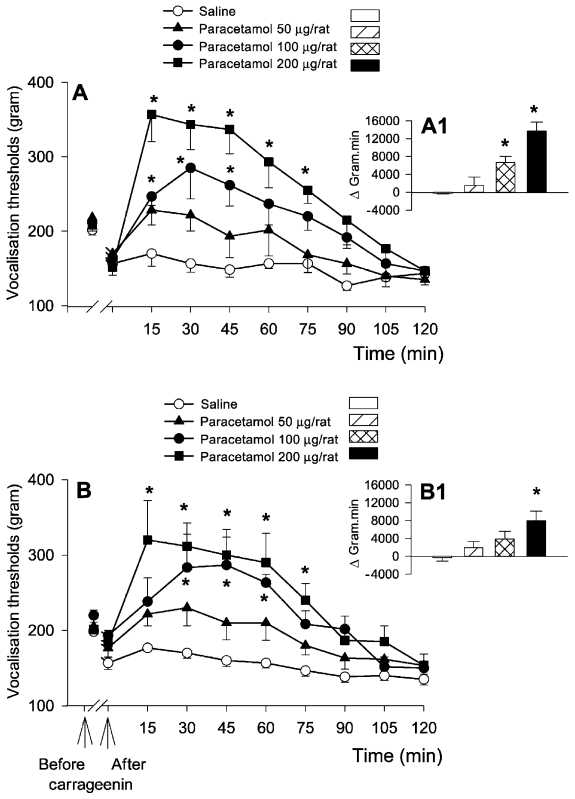

Fig. 2. Effect of intrathecal administration of paracetamol on the vocalisation thresholds to paw pressure in carrageenin-treated rats. Results are expressed (A, B) by the time-course curve of mean ± S.E.M. vocalisation thresholds (g) and (A1, B1) by the mean ± S.E.M. area under the time-course curves (AUC) of the variations (postdrug – predrug values of vocalisation thresholds), calculated by the trapezoidal method. Rats were treated with either saline or paracetamol (50, 100 and 200 µg/rat, i.t.) ($n=6$ per group) administrated 120 min after intraplantar injection of carrageenin. * $P<0.05$ vs. vehicle-treated group.

19.2 g (+145.8 ± 17.7%) for the carrageenin-injected paw (Fig. 1(A)) and +110.0 ± 9.5 g (+60.5 ± 5.7%) + 128.3 ± 26.0 g (+68.8 ± 10.7%) and +183.3 ± 28.8 g (+102.3 ± 17.6%) for the contralateral paw, respectively (Fig. 1(B)). The antinociceptive effect lasted 45 min on both hind paws. The determination of the AUC confirmed the significant antinociceptive effect of the all doses used without dose-dependency (Fig. 1(A1, B1)).

3.2. Antinociceptive effect of intrathecal injections of paracetamol

Intrathecally injected paracetamol (50, 100 and 200 µg/rat) also induced a significant ($P<0.05$) increase in vocalization thresholds. The maximum increases were +58.3 ± 20.0 g (+34.6 ± 11.8%), +121.6 ± 34.0 g (+72.2 ± 16.0%) and +205.0 ± 28.6 g (+134.7 ± 14.5%) for the carrageenin-injected paw (Fig. 2(A)) and +53.3 ± 22.6 g (+31.6 ± 12.2%), +93.2 ± 38.1 g (+49.3 ± 19.4%) and +136.6 ± 45.8 g (+71.2 ± 21.4%) for the contralateral paw, respectively (Fig. 2(B)). The effect was significant up to 45 to 75 min according to the dose used and to the paw tested. The expression of the values as AUC confirmed a dose-related antinociceptive effect only for the injured paw. In the non-injured paw, only the dose of 200 µg/rat induced a significant increase in AUC (Fig. 2(A1, B1)).

3.3. Influence of tropisetron on the antinociceptive effect of the paracetamol

The antinociceptive effect of paracetamol(200 mg/kg, i.v.) was confirmed (Figs. 3 and 4) as previously shown (Fig. 1), whatever the time of injection. However, the antinociceptive effect of pre-injury injection (Fig. 4) was significantly ($P=0.007$) more pronounced than that obtained when paracetamol was administered in the post-injury period (Fig. 3). Both maximal increases in vocal-

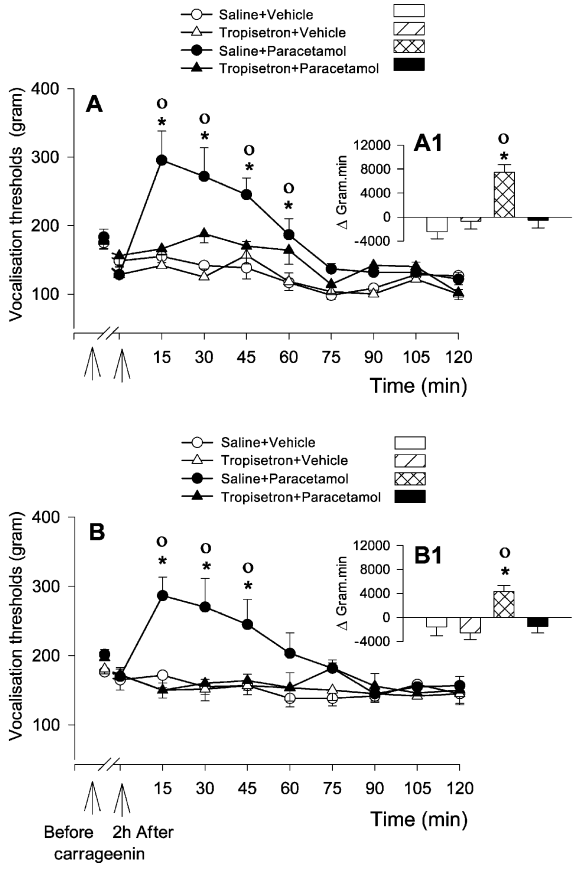

Fig. 3. Influence of tropisetron on the antinociceptive effect of paracetamol injected after carrageenin. Results are expressed (A, B) by the time-course curve of mean ± S.E.M. vocalisation thresholds (g) and (A1, B1) by the mean ± S.E.M. area under the time-course curves (AUC) of the variations (postdrug – predrug values of vocalisation thresholds), calculated by the trapezoidal method. Rats were treated with either saline (10 µl/rat, i.t.) or tropisetron (10 µg/rat, i.t.) injected 120 min after intraplantar injection of carrageenin. Vehicle (1 ml/kg, i.v.) or paracetamol (200 mg/kg, i.v.) were given 5 min after tropisetron. t15 min. corresponds to the first measurement after injection of paracetamol or vehicle. $n=6$ per group. * $P<0.05$ vs. saline + vehicle-treated group (A), ° $P<0.05$ vs. tropisetron + paracetamol-treated group (B).

isation thresholds (+233.3 ± 36.6 and +167.0 ± 40.3 g, respectively, for inflamed paw) and AUC (+16525.0 ± 3018.4 and +7455.0 ± 1311.0 g min, respectively) were significantly higher after paracetamol injected before than after injury.

Intrathecal injection of tropisetron (10 μg/rat), ineffective by itself, totally inhibited the activity of paracetamol either administered at post-injury (Fig. 3(A, B)) or pre-injury (Fig. 4(A, B)) time in both inflamed and non-inflamed paws. When results are expressed as AUC, the significant inhibition of the effect of paracetamol, post-injury administered, by tropisetron was 92.0 ± 19.1% ($P = 0.01$) (Fig. 3(A1)) and 90.7 ± 13.2% ($P < 0.01$) (Fig. 3(B1)) in the carrageenin-injected and the contralateral hind paw, respectively. Similar results were observed when paracetamol was used as a pre-injury treatment; inhibition was 119.2 ± 13.1% ($P < 0.001$) (Fig. 4(A1)) and 102.4 ± 8.8% ($P = 0.02$) (Fig. 4(B1)) in the carrageenin-injected and the contralateral hind paw, respectively.

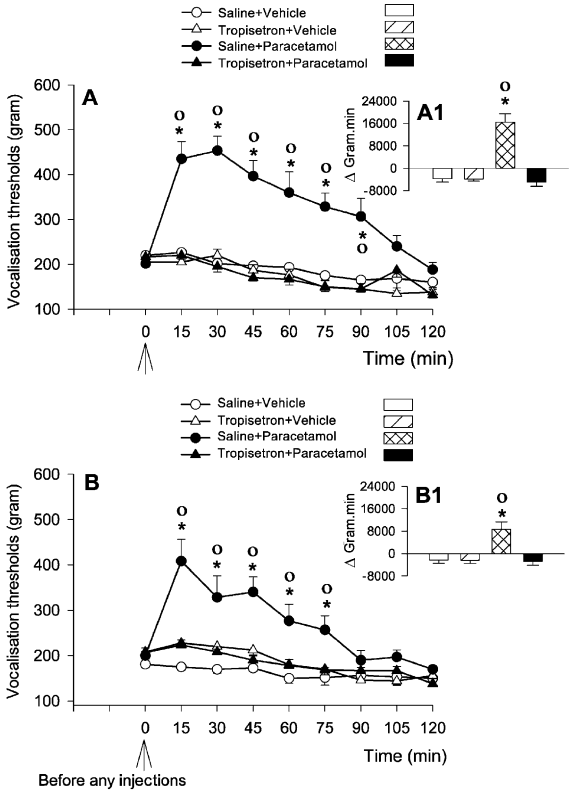

Fig. 4. Influence of tropisetron on the antinociceptive effect of paracetamol injected just before carrageenin. Results are expressed (A, B) by the time-course curve of mean ± S.E.M. vocalisation thresholds (g) and (A1, B1) by the mean ± S.E.M. area under the time-course curves (AUC) of the variations (postdrug − predrug values of vocalisation thresholds), calculated by the trapezoidal method. Five minutes after intrathecal injection of saline (10 μl/rat, i.t.) or tropisetron (10 μg/rat, i.t.), rats were treated intravenously with either vehicle (1 ml/kg) or paracetamol (200 mg/kg) followed 5 min later by intraplantar injection of carrageenin. t15 min corresponds to the first measurement after injection of paracetamol or vehicle. $n = 6$ per group. * $P < 0.05$ vs. saline + vehicle-treated group. ° $P < 0.05$ vs. tropisetron + paracetamol-treated group (A1, B1).

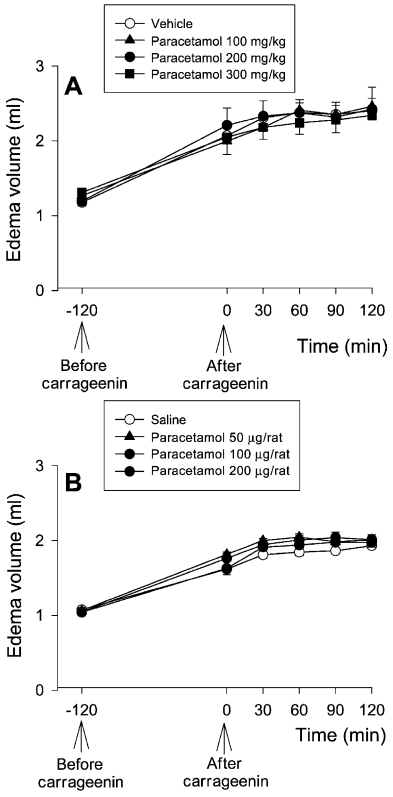

Fig. 5. Time-course of the action of paracetamol on the edema volume induced by intraplantar injection of carrageenin into the right hind paw ($n = 6$ per group). The volume of edema was expressed in ml (mean ± S.E.M.). Panels (A) and (B) represent the effect on edema of intravenous and intrathecal injections of paracetamol, respectively.

3.4. Influence of paracetamol on edema volume

Paracetamol, i.v. or i.t. administered 2 h after carrageenin, failed to induce any reduction in edema volume with or without treatment with tropisetron (Fig. 5). Similar results were observed when paracetamol was administered prior to carrageenin injection (data not shown).

4. Discussion

Paracetamol when injected i.v. after carrageenin induces a marked antinociceptive effect. It not only reverses mechanically induced hyperalgesia but also increases vocalization thresholds to values higher than pre-injury ones. This effect was dose-dependent only at the acme; that might be due to both the range of the doses used and the considerable ability of paracetamol to cross the blood brain (Courade et al., 2001a), which induces high and weakly different final concentrations at the site of action. Such an activity confirms the ability of paracetamol to reduce inflammation-induced pain both in experimental (Honoré et al., 1995) and in clinical (Seideman and Melander, 1988) conditions. However, the inflammatory mechanical hyperalgesia was

alleviated without any modification of edema, which indicates that paracetamol influences the nociceptive process differently from NSAIDs, which are able to reduce both hyperalgesia and inflammation (Randall and Selitto, 1957; Winter and Flataker, 1965; Honoré et al., 1995). This intrinsic antinociceptive effect of paracetamol has already been reported in non-inflammatory pain models in healthy animals (Pini et al., 1996; Pelissier et al., 1996; Sandrini et al., 1999).

Other data, obtained in the present work, tend to confirm the view that paracetamol does not act in the same way as NSAIDs. Pre-injury injection of paracetamol was effective and induced a more significant antinociceptive effect than post-injury administration. Conversely, aspirin is only effective when injected 24 h after carrageenin (Guilbaud et al., 1986). Moreover, in such a model of inflammatory pain, aspirin and NSAIDs only increase pain thresholds of the inflamed paw (Randall and Selitto, 1957) whereas, in our study, paracetamol induced a bilateral antinociceptive effect as do morphine and other opioids (Attal et al., 1990). Finally, in view of the chronology of the release of peripheral pronociceptive and proinflammatory mediators after injection of carrageenin, the anti-hyperalgesic effect of drug as rapidly effective as paracetamol and injected before carrageenin, cannot be due to a change in prostaglandin synthesis. During the first hour after the injection of carrageenin, inflammation is mediated by histamine and serotonin; kinins are released 1.5 h later, and prostaglandins and thromboxane appear only in a later phase, after the third hour (Di Rosa et al., 1971; Higgs and Salmon, 1979; Holsapple et al., 1980). An inhibitory effect of paracetamol on an NSAID-induced variant COX2-enzyme has been recently described by Botting (2000). However, the author surmises that this enzyme would be involved in the resolution of inflammation, i.e. in a late phase after carragenin administration, which disagrees with the involvement of such a mechanism in the ''rapid'' antinociceptive effect of paracetamol observed here, as in healthy rats (Pelissier et al., 1996). Hence, systemically administered paracetamol acts differently from aspirin and NSAIDs and independently of peripheral prostaglandin synthesis and of any anti-inflammatory effect.

Furthermore, the complete inhibition of antinociceptive effect of paracetamol by i.t. tropisetron, whatever the time (pre or post-injury) of its injection and thus the peripheral mechanisms involved in the initiation of hyperalgesia, demonstrates an involvement of the spinal 5-HT_3 receptors, even in conditions of inflammatory pain. This result is in line with findings reported elsewhere in healthy animals: i.t. tropisetron suppresses the effect of paracetamol in rats submitted to an electrical stimulus (Pelissier et al., 1995); the destruction of serotonin bulbospinal pathways inhibits the antinociceptive effect of paracetamol (Tjolsen et al., 1991). The precise nature of the interaction between paracetamol and the serotonergic system remains to be elucidated. The lack of affinity ($IC_{50} > 10^{-5}$ M) of paracetamol for 5-HT_3 and other 5-HT receptors (Raffa and Codd, 1996; Pelissier et al., 1996) tends to exclude any direct interaction with these targets. The demonstration by Pini et al. (1996) of an increase in central 5-HT levels would be in favor of an effect of paracetamol on the ''turnover'' of this monoamine. In a recent study, an increase in 5-HT release by paracetamol has been suggested (Courade et al., 2001a). However, tropisetron seems to possess some specificity in inhibiting 5-HT-induced antinociception (Bardin et al., 1997). For instance, low doses of tropisetron and granisetron were unable to inhibit the effect of a 5-HT_3 receptor agonist, and had a different influence on the effect of 5-HT since tropisetron blocked the effect of the monoamine while granisetron failed to do so. This different profile would suggest the existence of a specific non-5-HT_3 target, common to tropisetron and 5-HT. The nature of this target remains to be elucidated as well as the potential interaction with paracetamol.

In conclusion, paracetamol, when systematically administered, produces a spinally mediated antinociceptive action both in conditions of acute pain tests and in an inflammatory pain model without inducing any anti-inflammatory effect. In this respect, paracetamol can be distinguished from NSAIDs, thereby giving a new justification for using the combination of the two drugs, as usually performed in post-operative pain. Further experiments to elucidate the relationship of paracetamol with the serotonergic system, and to determine its molecular target in the central nervous system deserve investigation.

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