

# Presurgical ketoprofen, but not morphine, dipyron, diclofenac or tenoxicam, preempts post-incisional mechanical allodynia in rats

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## Abstract

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The treatment of pain before it initiates may prevent the persistent pain-induced changes in the central nervous system that amplify pain long after the initial stimulus. The effects of pre- or postoperative intraperitoneal administration of morphine (2 to 8 mg/kg), dipyron (40 and 80 mg/kg), diclofenac (2 to 8 mg/kg), ketoprofen (10 and 20 mg/kg), and tenoxicam (10 and 20 mg/kg) were studied in a rat model of post-incisional pain. Groups of 5 to 8 male Wistar rats (140-160 g) were used to test each drug dose. An incision was made on the plantar surface of a hind paw and the changes in the withdrawal threshold to mechanical stimulation were evaluated with Von Frey filaments at 1, 2, 6 and 24 h after the surgery. Tenoxicam was given 12 or 6 h preoperatively, whereas the remaining drugs were given 2 h or 30 min preoperatively. Postoperative drugs were all given 5 min after surgery. No drug abolished allodynia when injected before or after surgery, but thresholds were significantly higher than in control during up to 2 h following ketoprofen, 6 h following diclofenac, and 24 h following morphine, dipyron or tenoxicam when drugs were injected postoperatively. Significant differences between pre- and postoperative treatments were obtained only with ketoprofen administered 30 min before surgery. Preoperative (2 h) intraplantar, but not intrathecal, ketoprofen reduced the post-incisional pain for up to 24 h after surgery. It is concluded that stimuli generated in the inflamed tissue, rather than changes in the central nervous system are relevant for the persistence of pain in the model of post-incisional pain.

### Key words

- Preemptive analgesia
- Morphine
- Dipyron
- Diclofenac
- Ketoprofen
- Tenoxicam

## Introduction

Injury to peripheral tissues produces sensory changes such as prolonged pain, increased sensitivity to painful stimuli (hyperalgesia) and/or pain following innocuous stimulation (1). The phenomenon is accompanied by enlargement of the peripheral field

and increased excitability of spinal nociceptive cells in response to peripheral stimulation (2). A current view admits that excitatory amino acids activating NMDA receptors in the spinal cord produce excessive cell depolarization that contributes to increased pain sensation (3). According to this hypothesis, the treatment of pain before it initiates

would prevent the changes in the central nervous system, thus avoiding the amplification of pain long after the initial stimulus (2). Some studies have reported the efficacy of such "preemptive analgesia" following presurgical administration of opioids or local anesthetics to laboratory animals (4-6).

Several studies have shown that presurgical local or spinal anesthesia, or the systemic administration of analgesics can significantly reduce postsurgical pain (reviewed in 7). However, the intensity of pain produced by herniorrhaphy (8) or cholecystectomy (9) did not differ in groups of patients submitted to local infiltration of a local anesthetic before or soon after the end of the surgery. Also, no difference in postsurgical pain was detected when patients were treated with caudal or epidural block, or systemic administration of opioids before or after surgery (reviewed in 7). Reasons for the conflicting results include the use of postoperative pain measures and intraoperative administration of opioids as part of the general anesthesia procedures. Another possibility is that the pain perhaps depends more on postsurgical peripheral inflammation than on central sensitization that might develop during surgery (7,10,11).

Previous intrathecal (*it*) administration of lidocaine to rats prevents the pain response to formalin and reduces the expression of c-Fos protein in the spinal cord but is ineffective when administered after formalin (12). The prolonged nerve block with a local anesthetic prevented the late inflammatory hyperalgesia evoked by chronic intraplantar (*ipl*) administration of carrageenin in rats (13). These findings somewhat reinforce the notion that stimuli generated in the inflamed tissue may be relevant for the persistence of postoperative pain.

The present study was therefore undertaken to comparatively evaluate the effects of pre- or postoperative systemic administration of morphine (used as an opioid standard), dipyron (a nonsteroidal analgesic with

weak anti-inflammatory activity), and diclofenac, ketoprofen and tenoxicam (nonsteroidal analgesics with strong anti-inflammatory activities but different pharmacokinetic profiles) in a model of post-incisional pain in rats (14). Among the drugs studied, ketoprofen was the only one exhibiting preemptive analgesic efficacy in the model of postoperative pain.

## Material and Methods

The experiments were conducted on male Wistar rats (140-160 g) housed two to a cage with free access to food and water and maintained at an average ambient temperature of 24°C with a 12-h light-dark cycle before and after surgery. The proposals of the Committee for Research and Ethical Issues of the International Association for the Study of Pain (IASP) were followed throughout the experiments.

Each animal was anesthetized with 2% halothane via the nose cone. The plantar aspect of the right hind paw was prepared in a sterile manner with a 10% povidone-iodine solution. A 1-cm longitudinal incision was made with a surgical blade through the skin and fascia of the plantar region, starting 0.5 cm from the proximal edge of the heel. The plantaris muscle was elevated, but its origin and insertion were left intact. After hemostasis, the skin was joined with two 5-0 nylon sutures. After these procedures, the animal was allowed to recover in the home cage for a period of at least 1 h.

Mechanical threshold was measured with flexible nylon Semmes-Winstein Von Frey filaments (Stoelting, Wood Dale, IL, USA). Rats were placed in an elevated clear plastic cage with a nylon mesh bottom, which allowed easy access to the paw plantar surface. Before the test procedures, the animals remained in the cage for approximately 15 min for behavioral accommodation. The area tested was that adjacent to the wound on the mid-plantar right hind paw and the mechan-

ical thresholds were determined 1, 2, 6 and 24 h after surgery. The paw was touched with one of a series of nine Von Frey filaments with logarithmically incremental stiffness (0.69 to 75.858 g, lower and upper limit of the test, respectively). Each filament was applied from underneath the nylon mesh floor, through the mesh, vertically to the plantar surface with sufficient force to bend the filament a little. A single trial consisted of six applications of a particular filament, applied once every 3–4 s. Testing was initiated with the 3.63 g filament in the middle of the series. A response was defined as a withdrawal of the stimulated paw. In the absence of a response to a particular filament, the next stronger filament was utilized; in the case of a response, the next weaker filament was presented. The up-down method was used for threshold recording (15).

Different groups of rats were used for *ip* or *it* administration of drug or saline. In these animals the injections were performed 2 h before surgery. Intraplantar injections were given in a volume of 0.1 ml/paw and *it* injections were given in a volume of 10  $\mu$ l/rat using a method described elsewhere (16). In these animals, the withdrawal thresholds to mechanical stimulation were measured 2, 6 and 24 h after surgery.

The following drugs were used: morphine sulfate, dipyron, tenoxicam, and ketoprofen were from Sigma-Aldrich Co. (St. Louis, MO, USA); diclofenac sodium was from Ciba-Geigy (Basel, Switzerland). All drugs were diluted in saline. Tenoxicam was administered by the intraperitoneal (*ip*) route 12 or 6 h before, or 5 min after surgery. The remaining drugs were administered by the same route 2 h or 30 min before, or 5 min after surgery. Rats treated with drug-free saline (1 ml/kg) at the corresponding times before or after surgery were used as control. All doses in the text refer to the salt.

The results are reported as median with its corresponding confidence limits (95%) and are presented in graphs as means  $\pm$  SEM (in a

logarithmic scale) against time after surgery. The experimental groups were compared by the nonparametric Kruskal-Wallis test. Multiple comparisons after the Kruskal-Wallis test were performed using the two-tailed Dunn test. The level of significance was set at  $P < 0.05$ .

## Results

Before surgery, the rats did not exhibit a withdrawal reflex even during application of the thicker filament (75.858 g), taken here as the upper limit of the test. Similar results were obtained when applying the filaments against the plantar aspect of the non-operated hind paw (not shown in figures). In control rats the mean ( $\pm$  SEM) withdrawal thresholds measured 1 h after surgery decreased from the upper limit to  $0.461 \pm 0.08$ ,  $0.517 \pm 0.12$ , and  $0.459 \pm 0.15$  g for groups of animals that had received *ip* injections of saline 2 h or 30 min before, or 5 min after surgery, respectively (Figure 1, open bars).

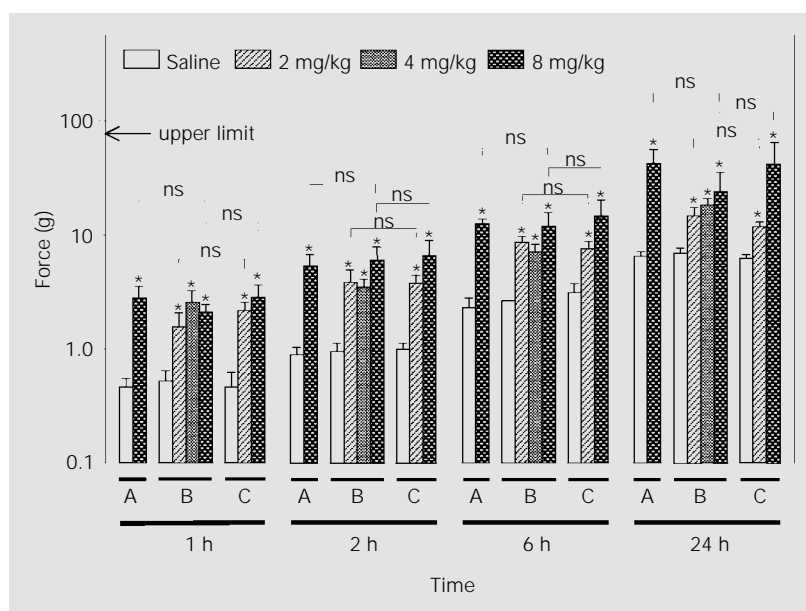


Figure 1. Changes produced by morphine in the post-incisional paw withdrawal thresholds in response to mechanical stimuli in rats. Morphine was administered intraperitoneally 2 h (A) or 30 min (B) before, or 5 min (C) after the incision. Times after surgery are indicated as hours. The upper limit (arrow) represents the maximum force (75.858 g) used in the test. Bars are means ( $\pm$  SEM) for 5 to 8 rats per group. \* $P < 0.05$  compared to control;  $P > 0.05$  (ns) for comparisons indicated by brackets (Dunn test).

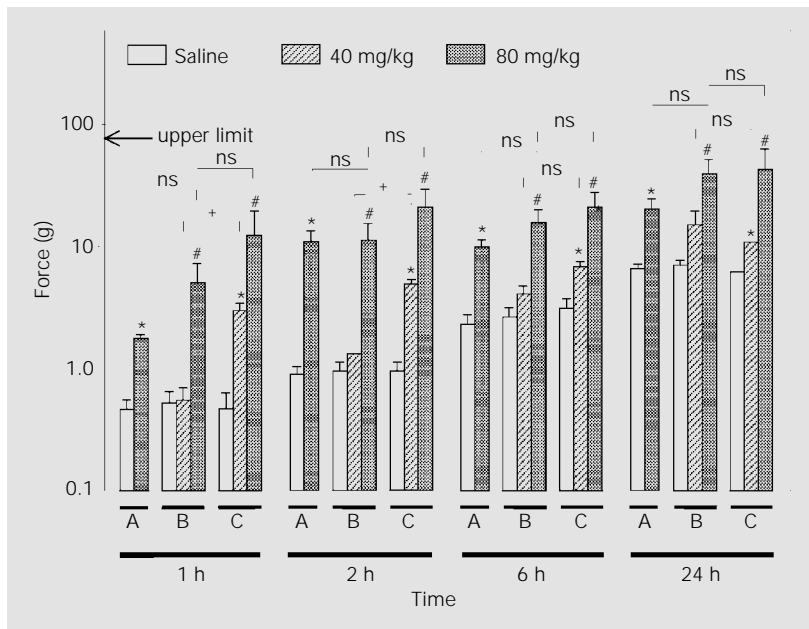


Figure 2. Changes produced by dipyron in the post-incisional paw withdrawal thresholds in response to mechanical stimuli in rats. Dipyron was administered intraperitoneally 2 h (A) or 30 min (B) before, or 5 min (C) after the incision. Times after surgery, the upper limit (arrow), and bars are as in Figure 1. \*P<0.05 compared to control; #P<0.05 compared to the remaining bars for the same time point and treatment. +P<0.05 and P>0.05 (ns) for comparisons indicated by brackets (Dunn test).

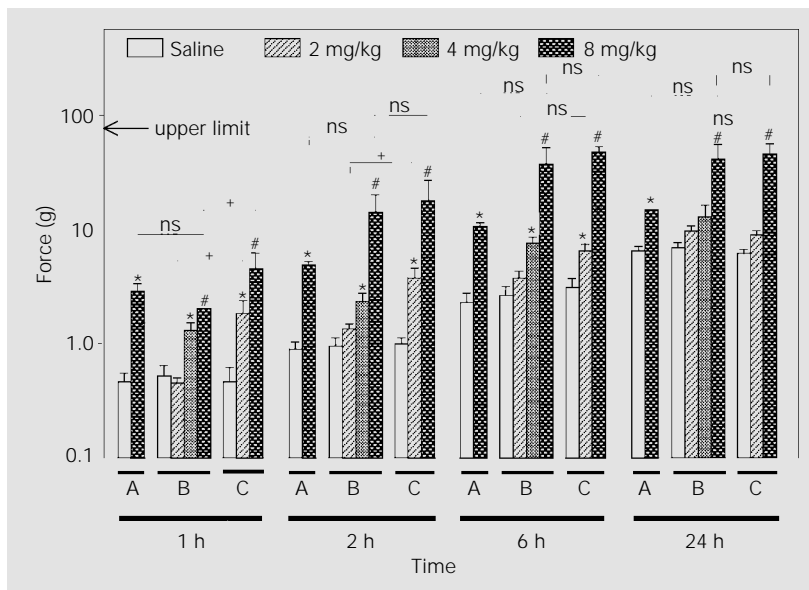


Figure 3. Changes produced by diclofenac in the post-incisional paw withdrawal thresholds in response to mechanical stimuli in rats. Diclofenac was administered intraperitoneally 2 h (A) or 30 min (B) before, or 5 min (C) after the incision. Times after surgery, the upper limit (arrow), and bars are as in Figure 1. \*P<0.05 compared to control; #P<0.05 compared to the remaining bars for the same time point and treatment. +P<0.05 and P>0.05 (ns) for comparisons indicated by brackets (Dunn test).

We found no significant differences in the withdrawal thresholds obtained on the four different occasions among the three control groups. In agreement with a previous report (14), a gradual return towards pre-incision values occurred over time. However, the withdrawal thresholds in all control groups remained below 8 g and were significantly different from pre-incision values throughout the postoperative period of observation.

Morphine (2 to 8 mg/kg) injected 2 h or 30 min before, or 5 min after surgery reduced, but did not abolish, the post-incisional mechanical allodynia (Figure 1). In each experimental group the thresholds of morphine-treated animals were significantly different from those of saline-treated rats at all times of observation. The effects of morphine (8 mg/kg) injected 2 h or 30 min before, or 5 min after surgery were not significantly different at any time of observation. Also, the effects of morphine (2 mg/kg) injected 30 min before or 5 min after surgery were not significantly different.

Dipyron (40 and 80 mg/kg) also reduced, but did not abolish, the post-incisional mechanical allodynia when injected 2 h or 30 min before, or 5 min after surgery (Figure 2). In the group treated 30 min before surgery, only rats that received the higher dose of dipyron had thresholds significantly different from the corresponding control. The effects of dipyron (80 mg/kg) injected 30 min before surgery did not differ significantly from the effect produced by the same dose administered 2 h before, or 5 min after surgery at any time of observation. The effects of dipyron (40 and 80 mg/kg) injected 5 min after surgery were significantly different from the corresponding control throughout the period of observation, but the differences between test and control thresholds decreased progressively at the later times of observation. In addition, the anti-allodynic effects obtained 1 or 2 h, but not 6 or 24 h, before surgery in rats pretreated with the lower dose of dipyron were significantly

lower than those produced by injections made after surgery.

Diclofenac (2 to 8 mg/kg) also reduced, but did not abolish, the post-incisional mechanical allodynia when injected 2 h or 30 min before, or 5 min after surgery (Figure 3). The effects of diclofenac (2 mg/kg) injected 5 min after surgery were significantly different from the corresponding control for up to 6 h after surgery. The effects of diclofenac (8 mg/kg) injected 2 h or 30 min before surgery were not significantly different at any time of observation. The effects of diclofenac (2 mg/kg) injected 30 min before or 5 min after surgery were significantly different only at 1 and 2 h. The effects of the highest dose of diclofenac injected 30 min before or 5 min after surgery were significantly different only at 1 h.

Ketoprofen (10 and 20 mg/kg) reduced, but did not abolish the post-incisional mechanical allodynia in all experimental groups (Figure 4). The effects of ketoprofen injected 5 min after surgery were significantly different from the corresponding control only at 1 and 2 h after surgery. In contrast, rats pretreated with ketoprofen 2 h or 30 min before surgery had significantly different thresholds compared to the corresponding control throughout the period of observation. The anti-allodynic effect of ketoprofen injected after surgery was significantly different from that obtained in rats treated 2 h before surgery only at 1 h. The anti-allodynic effect of ketoprofen injected 2 h before surgery was significantly different from that obtained in rats treated 30 min before surgery only at 1 and 2 h. The anti-allodynic effects of postoperative ketoprofen (20 mg/kg) at 2, 6 and 24 h after surgery were significantly weaker than those for rats treated 30 min before surgery. In contrast, the effects of postoperative ketoprofen (10 mg/kg) were not significantly different from those obtained in rats treated 30 min before surgery at all times.

Tenoxicam (10 and 20 mg/kg) also reduced, but did not abolish, the post-incisional mechanical allodynia in all experimental groups (Figure 5). The effects of tenoxicam

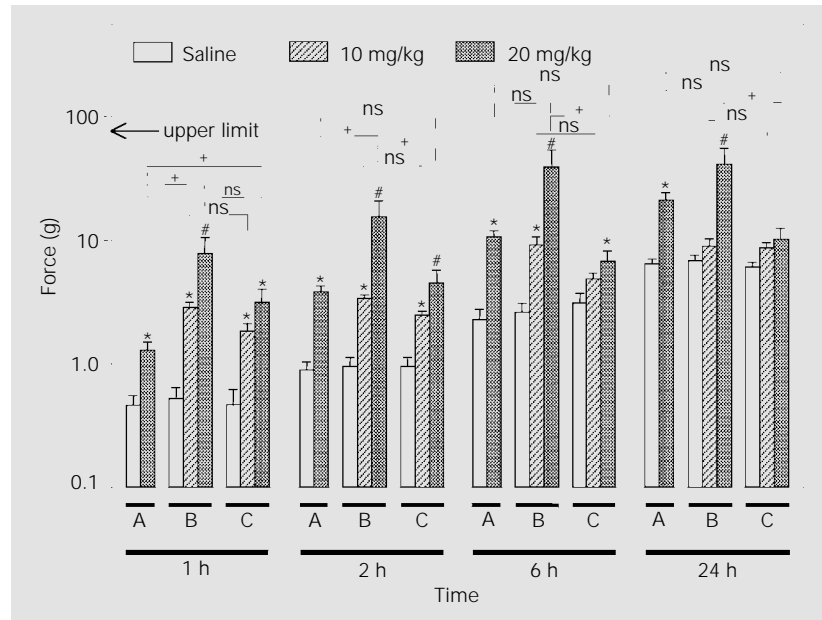


Figure 4. Changes produced by ketoprofen in the post-incisional paw withdrawal thresholds in response to mechanical stimuli in rats. Ketoprofen was administered intraperitoneally 2 h (A) or 30 min (B) before, or 5 min (C) after the incision. Times after surgery, the upper limit (arrow), and bars are as in Figure 1. \* $P < 0.05$  compared to control; # $P < 0.05$  compared to the remaining bars for the same time point and treatment. \* $P < 0.05$  and  $P > 0.05$  (ns) for comparisons indicated by brackets (Dunn test).

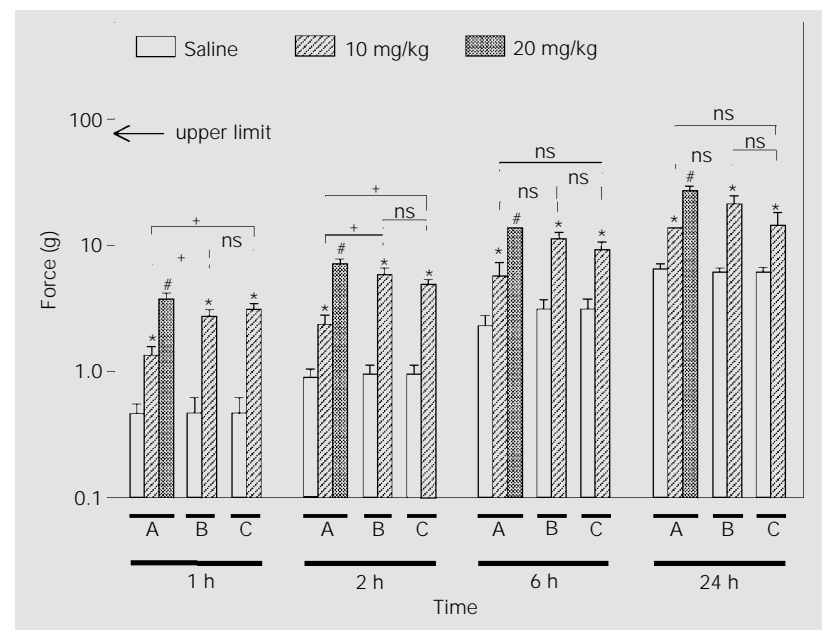


Figure 5. Changes produced by tenoxicam in the post-incisional paw withdrawal thresholds in response to mechanical stimuli in rats. Tenoxicam was administered intraperitoneally 12 h (A) or 6 h (B) before, or 5 min (C) after the incision. Times after surgery, the upper limit (arrow), and bars are as in Figure 1. \* $P < 0.05$  compared to control; # $P < 0.05$  compared to the remaining bars for the same time point and treatment. \* $P < 0.05$  and  $P > 0.05$  (ns) for comparisons indicated by brackets (Dunn test).

(10 mg/kg) injected 5 min after surgery were significantly different from the corresponding control throughout the period of observation, but the differences between test and control thresholds were progressively smaller at later times of observation. The effects of postoperative administration of tenoxicam (10 mg/kg) observed at 1 and 2 h also differed significantly from those produced in rats injected with tenoxicam 12 h before surgery but did not differ significantly from those obtained for rats treated 6 h before surgery. The effects of tenoxicam injected 12 h before surgery were significantly lower than those obtained at times 1 and 2 h for animals injected with tenoxicam 6 h before surgery.

Rats treated 2 h before surgery with *ipl* ketoprofen (2 and 10 mg/kg) (Figure 6A) showed a significant and dose-dependent reduction of mechanical allodynia throughout the period of observation. For compari-

son, the effects of ketoprofen (2 mg/kg) injected by the *ip* route 2 h before surgery were not significantly different from control. In contrast, *it* ketoprofen (100 µg/kg) injected 2 h before surgery significantly reduced the mechanical allodynia only at 2 and 6 h, whereas the smaller dose (10 µg/kg) had no effect (Figure 6B).

## Discussion

In the present study we have examined whether morphine, dipyrone, diclofenac, ketoprofen, and tenoxicam produce “pre-emptive analgesic” effects in a rat model of post-incisional pain. At the doses used here, no drug completely abolished the post-incisional mechanical allodynia when injected before or 5 min after surgery. However, the withdrawal thresholds were significantly higher than in control rats for up to 2 h following ketoprofen, 6 h following diclofenac, and 24 h following morphine, dipyrone or tenoxicam when drugs were injected postoperatively.

Except for the smaller doses of dipyrone or diclofenac, all other preoperative treatments reduced the post-incisional allodynia when compared to saline-treated rats. The withdrawal thresholds for rats treated postoperatively with morphine, dipyrone, diclofenac, or tenoxicam were always higher than for rats treated preoperatively. Significant differences between pre- and postoperative treatments were found at times 1 and 2 h only in experiments in which drugs were injected 30 min (lower doses of dipyrone or diclofenac) or 6 h (tenoxicam) before surgery. Significant differences between pre- and postoperative treatments were also found at times 1 h only for experiments in which the highest dose of diclofenac was injected 30 min before surgery. At the doses used here, postoperative ketoprofen had a weak and short-lasting effect against the post-incisional mechanical allodynia. However, a significant reduction in the withdrawal threshold

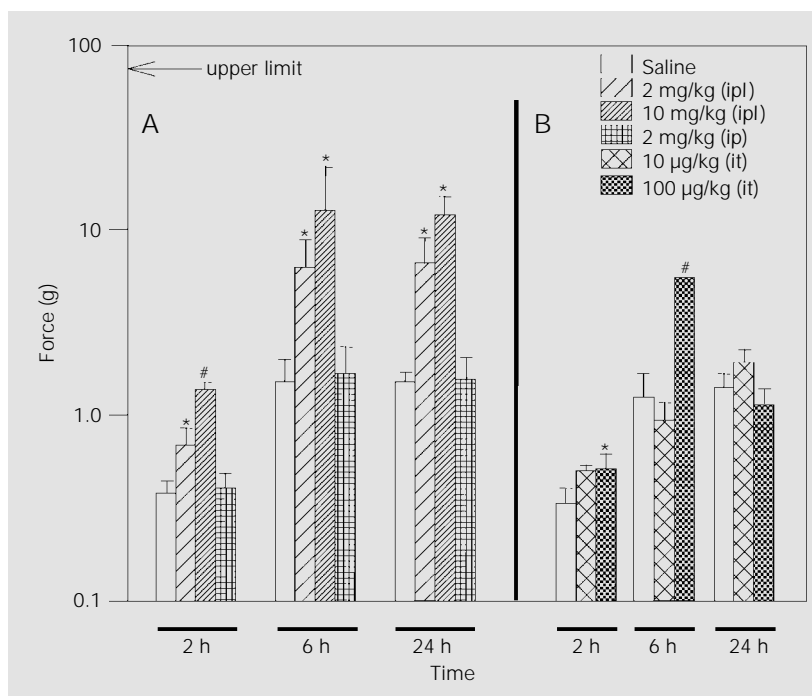


Figure 6. Changes produced by ketoprofen in the post-incisional paw withdrawal thresholds in response to mechanical stimuli in rats. Ketoprofen was administered by the intraplantar (*ipl*), intraperitoneal (*ip*) (A), or intrathecal (*it*) route (B) 2 h before the incision. Times after surgery, the upper limit (arrow), and bars are as in Figure 1. \* $P < 0.05$  compared to control; # $P < 0.05$  compared to the remaining bars for the same time point and treatment (Dunn test).

was present for up to 24 h in groups of rats treated with ketoprofen (10 or 20 mg/kg) 30 min before surgery.

“Preemptive analgesia” is an antinociceptive treatment applied before surgery that prevents the establishment of altered processing of afferent input, which amplifies postoperative pain (17) and, therefore, covers the period of surgery and the initial postoperative period (18). Some authors consider that “preemptive analgesia” should prevent nerve impulses generated by the injury from reaching and sensitizing neurons in the spinal cord (18). Thus, a “preemptive analgesic” should provide a more effective reduction in incisional allodynia than an analgesic administered postoperatively (19). In the present study, only preoperative ketoprofen (20 mg/kg) produced better pain relief than when administered postoperatively and, therefore, was the only one fulfilling the criteria for a “preemptive analgesic” in the model of post-incisional pain. However, we found no preemptive analgesic efficacy for ketoprofen administered 2 h before surgery.

Morphine was ineffective as a preemptive analgesic in the model of post-incisional pain following *ip* administration. Intrathecal morphine was also ineffective in the same model (19). In addition to its well-known central effects, morphine may also act peripherally on inflamed tissue (20). We may thus conclude that neither peripheral nor spinal opioid mechanisms are involved in the generation of post-incisional pain in the model.

Nonsteroidal anti-inflammatory drugs (NSAIDs), including dipyron, diclofenac, ketoprofen, and tenoxicam, are inhibitors of cyclooxygenase enzyme, thus reducing the generation of prostaglandins that sensitize peripheral nociceptors (reviewed in 21). In addition to inhibiting cyclooxygenase, diclofenac (22) and dipyron (23) also down-regulate sensitized peripheral pain receptors. In fact, dipyron possesses anti-inflammatory-independent antinociceptive activity

in the mouse formalin, hot-plate and tail-flick tests that depends on peripheral and central sites of action (24), and has a significant antinociceptive effect even in the absence of an anti-inflammatory response (25). In patients undergoing third molar extraction, there are reports for (26) and against (27) a preemptive analgesic property of dipyron or diclofenac.

Tenoxicam has a longer half-life (60 h) than ketoprofen and diclofenac (28,29) and produces strong analgesia that occurs very rapidly. Tenoxicam-induced preemptive analgesia has been demonstrated for the control of postoperative pain of patients submitted to major gynecological surgery (30) or patients undergoing a breast biopsy (31). In the present study, however, we found no preemptive analgesic effectiveness for tenoxicam administered either 12 or 6 h before surgery. Thus, the timing of drug administration is unlikely to be a prerequisite for preemptive analgesia.

Herrero et al. (32) reported that ketoprofen was effective in the reduction of reflex wind-up, a phenomenon in which the activity of spinal cord neurons is increased with high frequency of peripheral electrical stimulation. The effect, however, was obtained only with doses of ketoprofen 4 to 16 times higher than those used in the present study. We found *it* ketoprofen to be effective against post-incisional pain when injected 2 h before surgery, but the effect lasted up to 6 h only. Therefore, inhibition of spinal wind-up is unlikely to be the mechanism of the preemptive analgesic efficacy of ketoprofen. Ketoprofen (1 to 10 mg/kg) reduced in a dose-dependent manner the local edema and expression of c-Fos-like immunoreactivity in spinal neurons following later administration of carrageenan in the rat hind paw, but was ineffective against the increase in the expression of spinal c-Fos-like immunoreactivity induced by noxious heat stimulation (33). These results were taken as evidence for a predominant anti-inflammatory, and

not potent analgesic effectiveness of ketoprofen, acting at a peripheral site. In agreement with this view, we found that *ipl*, but not *ip*, ketoprofen was highly effective in reducing post-incisional allodynia throughout the period of observation when injected 2 h before surgery. Ketoprofen differs from the other NSAIDs because, in addition to inhibiting cyclooxygenase, it also inhibits the lipoxygenase pathway, thus preventing the generation of both prostaglandins and leukotrienes (reviewed in 34). Leukotrienes are known to produce hyperalgesia in animals (35) and humans (36), and seem to play an important role in the maintenance of long-lasting nociceptive responses (37).

The present study demonstrates that pre-operative *ip* ketoprofen, but not morphine, dipyron, diclofenac or tenoxicam reduces post-incisional pain in rats. This finding supports the view that stimuli generated in inflamed tissue involving both the cyclooxygenase and lipoxygenase cascades are relevant for the persistence of postoperative pain.

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