



# Intrathecal morphine and ketorolac analgesia after surgery: comparison of spontaneous and elicited responses in rats

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## Abstract

Pain after surgery results in significant morbidity, and systemic opioids often fail to provide adequate analgesia without marked sedation and respiratory depression. Intrathecal morphine provides better analgesia, but is limited by delayed respiratory depression. Intrathecal injection of the cyclooxygenase inhibitor, ketorolac, has recently entered clinical trials, and the current study examined the interaction between intrathecal morphine and ketorolac to treat postoperative pain. We also sought to compare these treatments on a commonly used assessment of withdrawal threshold and a new assessment of spontaneous behavior after surgery. Male Sprague Dawley rats and underwent hind paw incision or subcostal laparotomy surgery. Intrathecal morphine, ketorolac, or their combination were injected on the first postoperative day, with outcome measure being return to pre-surgery withdrawal threshold with von Frey filament testing of the paw after paw incision, or return to pre-surgery exploratory activity after laparotomy. Intrathecal morphine completely reversed the effects of surgery in both models, but intrathecal ketorolac only partially reversed them. Ketorolac enhanced the potency of morphine several fold in both models, and did so synergistically after paw incision. In all cases drug potency was greater for spontaneous than elicited responses. These data confirm that spinal opioid receptor and cyclooxygenase enzyme inhibition diminish elicited tactile hypersensitivity after surgery, and that they similarly return spontaneous behavior to normal. Differences in drug potency could reflect fundamental differences in outcome measures or in the surgical procedures themselves. These data support combination study of intrathecal morphine and ketorolac for postoperative pain.

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## 1. Introduction

Increased severity of postoperative pain is associated with increased morbidity (Carli and Bennett, 2001) and with increased risk of development of chronic pain (Perkins and Kehlet, 2000). Despite educational, organizational, and pharmacological developments, postoperative pain remains poorly treated, with nearly half of patients experiencing severe pain in the postoperative period, and there has been

little improvement in this incidence over the last three decades (Apfelbaum et al., 2003).

One purpose of the current study was to examine the interaction between intrathecal morphine and ketorolac for postoperative pain. Noxious stimuli increase prostaglandin synthesis in the spinal cord (Ramwell et al., 1966), and case reports 20 years ago suggested that intrathecal injection of cyclooxygenase (COX) inhibitors produced analgesia in humans (Devoghel, 1983). In rats, intrathecal injection of COX inhibitors reduces tactile hypersensitivity following incision of the hind paw, a model of postoperative pain (Zhu et al., 2003). Previous studies demonstrate a synergistic interaction between intrathecal morphine and COX inhibitors to reduce intraplantar formalin induced behaviors (Malmberg and Yaksh,

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1993), and to reduce tactile hypersensitivity after paw incision (Kroin et al., 2002). However, the latter used a COX-2 selective inhibitor, and these are inactive after intrathecal injection after paw incision (Kroin et al., 2002; Yamamoto and Sakashita, 1999), whereas, the COX-1 preferring inhibitor, ketorolac, is active (Zhu et al., 2003). For this reason, and since ketorolac has completed neurotoxicity studies (Yaksh et al., 2004) and is in clinical trials (Eisenach et al., 2002), its interaction with morphine deserves study. We hypothesized that intrathecal ketorolac would synergistically enhance the effect of intrathecal morphine after surgery.

Another purpose of the current study was to compare the effects of these drugs on spontaneous behavior and on withdrawal response to tactile stimulation with von Frey filaments. Although commonly used to assess hypersensitivity, the validity of withdrawal threshold to von Frey filament probing as a measure of allodynia or pain after nerve injury has been recently questioned (Hogan et al., 2004). Doses of aspirin which are analgesic in humans do not affect withdrawal threshold to von Frey filament testing in rats with peripheral inflammation, yet they do affect more complex escape avoidance behavior (LaBuda and Fuchs, 2001). We recently described a method to assess exploratory behavior in rodents, demonstrating that this was disrupted after laparotomy surgery (Martin et al., 2004). Systemic morphine returned the disrupted behavior towards normal, although it was ineffective to restore rearing behavior, during which the animal stretched the abdominal musculature. Systemic ketorolac alone had minor effects, but enhanced that of morphine. In the current study, we hypothesized that intrathecal morphine would be more effective, returning even the rearing behavior to normal. Since spinal prostaglandins are especially important to sensitized states, we further hypothesized that intrathecal ketorolac would be more potent and effective to treat tactile hypersensitivity than to alter spontaneous behavior.

## 2. Materials and methods

### 2.1. Animals and surgical preparation

#### 2.1.1. Common procedures

Following approval by the Animal Care and Use Committee, male Sprague–Dawley rats weighing 200–250 g (Harlan, Indianapolis, IN, USA.  $n=73$  for paw incision,  $n=336$  for laparotomy) were anesthetized and a 32G polyethylene catheter (ReCathCo, Allison Park, PA) connected to a piece of Tygon external tubing (Saint–Gobain Performance Plastics, Akron, OH) was inserted 7.5 cm (paw incision animals) or 6.0 cm (laparotomy animals) through the cisterna magnum as previously described (Yaksh and Rudy, 1976) until the tip lay near the lumbar enlargement. Rats showing neurologic deficits were euthanized. After surgery, animals were housed individually in plastic cages in a climate-controlled room under a 12–12 h light–dark cycle with free access

to food and water. At the end of behavioral experiments, some rats were intrathecally injected with 10  $\mu$ l of 2% lidocaine, and bilateral motor blockade taken as confirmation of correct catheter position. An intrathecal catheter was not inserted in all animals for laparotomy.

#### 2.1.2. Paw incision

One week after intrathecal catheterization, paw incision was performed as previously described (Brennan et al., 1996). Briefly, rats were anesthetized with halothane, and after sterile preparation with 70% ethanol, a 1-cm long incision was made in the plantar aspect of the left hind paw, starting 0.5 cm from edge of the heel toward the toe. The plantaris muscles were elevated and incised longitudinally. The wound was closed with two mattress sutures of 6.0 silk.

#### 2.1.3. Laparotomy

One week after intrathecal catheterization, laparotomy was performed as previously described (Martin et al., 2004). Briefly, rats were anesthetized with isoflurane, the right upper quadrant of the abdomen shaved, and a 3-cm incision made diagonally 0.5 cm below and parallel to the lowest rib, penetrating the peritoneal cavity. The wound was vigorously dilated by inserting 0.5 cm of the index finger into the peritoneal cavity, then the wound was closed in layers and dressed with antibiotic powder. Sham treated animals were anesthetized and shaved only.

### 2.2. Behavioral measurements

#### 2.2.1. Paw incision

Withdrawal threshold of the left paw was measured in response to application of von Frey filaments, using an up–down method to determine the 50% likelihood of withdrawal (Chaplan et al., 1994). Rats were placed individually in clear Plexiglas boxes above a plastic mesh floor, which allowed full access to the paws. Following acclimation to the environment for at least 20 min, von Frey hairs in log increments of force were applied for 5 s to the left mid-plantar paw. The withdrawal threshold was determined before (pre-surgery threshold) and 24 h after incision (baseline threshold) and then every 30-min for 4 h after intrathecal injection. In addition to withdrawal threshold testing, gross behavior including ambulation and activity level was observed after intrathecal injection.

#### 2.2.2. Laparotomy

Exploratory behavior was assessed 24 h after laparotomy, using commercially available equipment and software (Med Associates Inc., St Albans, VT) as previously described (Martin et al., 2004). Briefly, animals were placed in activity chambers equipped with duplicate banks of 16 infrared transmitters spaced 2.5 cm apart with aligned detectors on the opposing sides of the chamber. A third bank of infrared transmitters and detectors, located 7 cm above the floor surface, allowed determination of rearing behavior. Data were collected in 6 min bins for 1 h, and included total distance traveled in the X–Y plane, total beam breaks in the X–Y plane (ambulatory counts), repeated beam breaks within 3 cm of the animal in the absence of locomotion (stereotypy), and total beam breaks in the upper X direction (rearing). Studies were performed during the dark phase of the 12:12 h light:dark cycle.

### 2.3. Drug administration

Morphine sulfate was purchased from Astra Pharmaceutical Inc. (Westborough, MA). Ketorolac was purchased from Allergan Inc. (Irvine, CA). All drugs for intrathecal injection were made freshly right before the experiments. Each group consisted of six to ten rats. Dose ranges administered, determined from pilot experiments, were for morphine 0.25, 0.5, 0.75, and 1.5  $\mu\text{g}$  after paw incision and 0.003, 0.1, 0.3, 1, 3, and 10  $\mu\text{g}$  after laparotomy. For ketorolac, doses were 5, 25, 50, 150  $\mu\text{g}$  after paw incision and 5, 15, 30, and 50  $\mu\text{g}$  after laparotomy. For study of drug interactions, an isobolographic approach was employed with the paw incision model, using a fixed morphine:ketorolac ratio of 1:142 wt/wt and doses of 0.44+6.3, 0.13+18.8, and 0.44+62.5  $\mu\text{g}$  of morphine+ketorolac, respectively. For study of drug interactions in the laparotomy model, in which there were multiple outcome measures, it was not feasible to perform multiple isobolographic studies based on the drug's relative potencies for each outcome measure. For this reason, drug interactions were studied by combining an ineffective dose of ketorolac (5  $\mu\text{g}$ ) with a full dose range of morphine. All intrathecal injections were followed by a 10  $\mu\text{l}$  saline flush, and saline was used as vehicle. The investigator was blinded to drug treatment for all studies.

### 2.4. Data analysis

#### 2.4.1. Paw incision

For calculation of dose responses, paw withdrawal thresholds were converted to percentage maximal possible effect (%MPE) according to the formula:  $\%MPE = (\text{post-drug threshold} - \text{baseline threshold}) / (\text{pre-surgery threshold} - \text{baseline threshold}) \times 100$ . Paw withdrawal thresholds and %MPE were normally distributed. Sigmoidal nonlinear regression curve fitting for dose-response data was performed using Origin 6.0 software (OriginLab, Northampton, MA). Time-course and dose-response effects of morphine and ketorolac were analyzed using two-way ANOVA with Student-Newman-Keuls post hoc test. Drug interaction was analyzed by conventional isobolographic analysis (Tallarida et al., 1989), using an effect level that each drug alone was capable of achieving (dose to achieve a 20% MPE). The difference between the theoretical additive point and the experimentally determined value was compared by the Student's *t*-test.

#### 2.4.2. Laparotomy

Behavioral data were analyzed using one-way ANOVA on the incision group and the sham group separately. Post hoc analyses were performed using the Dunnett's *t*-test for multiple comparisons to a control with non-catheterized sham animals serving as the control group. Dose responses were analyzed by non-linear regression fitting as described above, using % return to non-catheterized sham animal activity as the measure.

All data are presented as mean  $\pm$  SE.  $P < 0.05$  was considered statistically significant. To compare drug potency between the two postoperative models, non-linear curve fitting was performed, determining the maximum effect ( $E_{\text{max}}$ ) and the dose producing 50% of the maximum effect (ED50).

## 3. Results

### 3.1. Paw incision

Paw incision resulted in a reduced paw withdrawal threshold 24 h after surgery compared with presurgical values ( $5.0 \pm 0.21$  g after vs.  $21 \pm 0.22$  g before;  $P < 0.01$ ;  $n = 73$ ), with no differences across treatment groups. Intrathecal administration of morphine produced a dose-dependent increase in withdrawal threshold, with a peak effect 30 min after injection (Fig. 1A). Ketorolac was less effective than morphine, but also dose-dependently increased withdrawal threshold, with a peak effect 30 min after injection (Fig. 1B). The fixed ratio combination of morphine and ketorolac also produces dose dependent increases in withdrawal threshold, with a peak effect 30 min after injection (Fig. 1C). Neither the administration of each drug alone nor their combination affected ambulation or the level of general activity following paw incision.  $E_{\text{max}}$  and ED50 values are listed in Table 1.

Doses producing a 20% MPE effect for morphine and ketorolac (with 95% confidence limits) were 0.34 (0.30–0.38) and 50 (33–84)  $\mu\text{g}$ , respectively, alone and 8.5 (5.4–12)  $\mu\text{g}$  (summed dose of each compound together) in combination (Fig. 2A). Isobolographic analysis indicated a synergistic interaction between morphine and ketorolac (Fig. 2B).

### 3.2. Laparotomy

Laparotomy decreased all parameters of exploratory behavior, as previously reported (Martin et al., 2004) (Figs. 3–7). Comparing sham and incision control groups (no i.t. catheter), the incision decreased the total distance traveled by  $42 \pm 10\%$  ( $[F(1,15) = 10.9, P = 0.005]$ ). All other behavioral parameters were significantly affected by the abdominal incision with ambulation, stereotypy and rearing being decreased by  $45 \pm 11$ ,  $25 \pm 8$  and  $32 \pm 12\%$  relative to the behavior recorded from sham control subjects, respectively [ambulation  $F(1,15) = 10.1, P = 0.007$ ; stereotypy  $F(1,15) = 7.3, P = 0.02$ ; rearing  $F(1,15) = 5.3, P = 0.04$ ]. Intrathecal catheterization produced a significant effect on exploratory behaviors in both sham and incision groups when compared to non-catheterized controls [total distance  $F(1,39) = 9.6, P = 0.004$ ; ambulation  $F(1,39) = 7.3, P = 0.1$ ; stereotypy  $F(1,39) = 5.5, P = 0.03$ ; rearing  $F(1,39) = 10.5, P = 0.003$ ]. The abdominal incision produced a similar effect on exploratory behavior in i.t. catheterized animals as in non-catheterized subjects however. Total distance traveled was decreased by  $38 \pm 7\%$  following abdominal incision compared to sham treatment in i.t. catheterized subjects [ $F(1, 23) = 11.0, P = 0.004$ ]. Ambulation, stereotypy and rearing were decreased by  $41 \pm 8$ ,  $24 \pm 6$  and  $35 \pm 13\%$ , respectively, following abdominal incision compared to sham-treatment in i.t. catheterized animals [ambulation  $F(1,23) = 8.4, P = 0.009$ ;

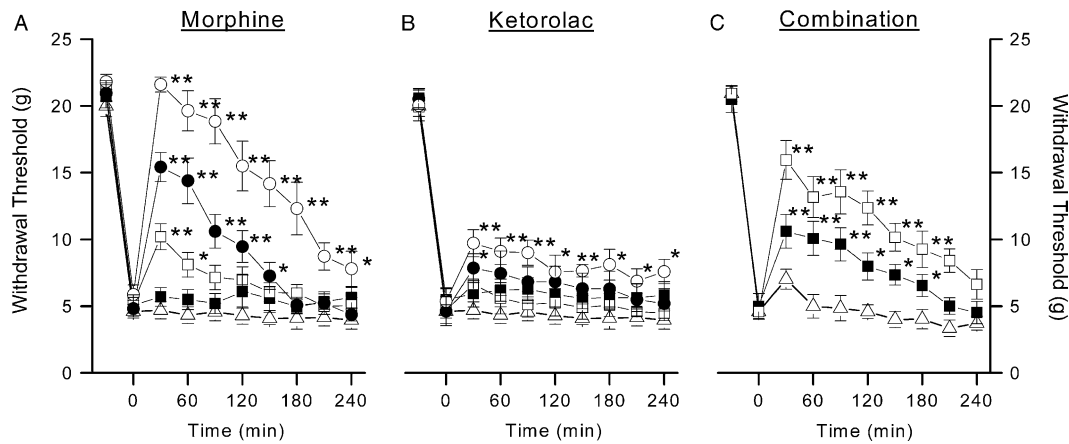


Fig. 1. Withdrawal threshold after intrathecal injection, at time 0, of morphine (A, left panel), ketorolac (B, middle panel), or a fixed ratio combination (C, right panel) to von Frey filament stimulation of the left hindpaw. Values prior to 0 represent those prior to paw incision. Saline is shown in open triangles, and increasing doses are shown in solid squares, open squares, solid circles, and open circles, respectively (doses listed in Methods). Each symbol represents the mean  $\pm$  SE of 6–10 animals. \* $P < 0.05$  compared to time 0. \*\* $P < 0.01$  compared to time 0.

stereotypy  $F(1,23)=7.6$ ,  $P=0.01$ ; rearing  $F(1,23)=9.5$ ,  $P=0.006$ ]. Comparing sham and incision groups between i.t. catheterized and non-catheterized subjects using a 2-way ANOVA, the effect of the abdominal incision on exploratory behavior was not found to be significantly different between catheterized and non-catheterized rats. There was no significant interaction between abdominal surgical treatment and i.t. catheterization vs. non-catheterization [total distance  $F(1,39)=1.2$ ,  $P=0.29$ ; ambulation  $F(1,39)=1.1$ ,  $P=0.30$ ; stereotypy  $F(1,39)=0.2$ ,  $P=0.66$ ; rearing  $F(1,39)=0.2$ ,  $P=0.70$ ]. Therefore, the abdominal incision decreased exploratory behaviors in rats in a similar manner and to a similar extent as our previous findings (Martin et al., 2004) in both i.t. catheterized and non-catheterized animals.

3.2.1. Effects of intrathecal morphine

Morphine attenuated the effects of abdominal surgery on exploratory behaviors in a dose-dependent manner and

completely reversed the effects of laparotomy with an optimum dose of 1  $\mu$ g (Figs. 3–7). Morphine produced an increase in exploratory behavior following laparotomy for distance traveled [ $F(8,90)=4.6$ ,  $P=0.0001$ ] with doses of 0.3 or 1.0  $\mu$ g producing an increase compared to saline administration and resulting in distance traveled that was not significantly different than the control sham group ( $P \leq 0.05$ ). The 10  $\mu$ g dose of morphine decreased distance traveled relative to the control sham group. The dose-response curve for morphine’s effect on ambulation was similar following abdominal incision [ $F(8,90)=3.4$ ,  $P=0.002$ ], with 0.3 and 1  $\mu$ g increasing behavior relative to saline administration and 10  $\mu$ g of morphine resulting in a decrease in ambulation relative to the control sham group ( $P \leq 0.05$ ). The dose-response curve for morphine’s effects on stereotypy following abdominal incision was somewhat more shallow, with all doses producing a significant elevation compared to saline administration [ $F(8,90)=4.1$ ,  $P=0.0004$ ]. The dose-effect curve for morphine’s effects on

Table 1  
Maximum efficacy and potency of morphine and ketorolac

Drug	$E_{max}$ (ED50) <sup>a</sup>				
	Withdrawal threshold	Distance	Ambulation	Stereotypy	Rearing
<i>Morphine</i>					
$E_{max}$ (%)	100	100	100	100	100
ED50 ( $\mu$ g)	0.63	0.081	0.085	0.038	0.0066
Ratio of ED50 to reverse effects on withdrawal threshold to ED50 to reverse effects on behavior	–	0.13	0.14	0.06	0.01
<i>Ketorolac</i>					
$E_{max}$ (%)	30	49	44	71	73
ED50 ( $\mu$ g)	32	8.4	14	5.7	8.3
Ratio of ED50 to reverse effects on withdrawal threshold to ED50 to reverse effects on behavior	–	0.26	0.44	0.18	0.26
Ratio of ED50 of ketorolac/ED50 of morphine	50	10	17	150	1300

<sup>a</sup>  $E_{max}$ , maximum efficacy, in % return to pre-surgery or control values. ED50, dose producing a 50%  $E_{max}$  effect level.

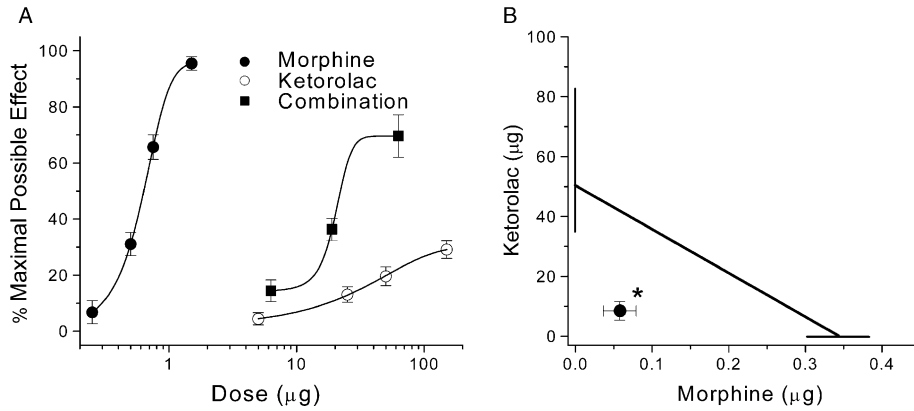


Fig. 2. (A) Dose response at the time of peak effect of intrathecal morphine, ketorolac, or a fixed ratio combination, expressed as % maximum possible effect to return withdrawal threshold to pre-paw incision values. Curves are fit by non-linear sigmoidal function. Each symbol represents the mean  $\pm$ SE of 6–10 animals. (B) Isobologram at the 20% maximum possible effect level. \* $P < 0.05$  compared to the theoretical additive point.

rearing following laparotomy was also rather shallow, with all doses lower than 1 μg producing a significant effect compared to saline [ $F(8,90) = 2.5$ ,  $P = 0.02$ ]. Administration of 3 or 10 μg of morphine resulted in decreases in

rearing behavior following incision compared to the control sham group. As noted above, intrathecal catheter placement significantly decreased exploratory behavior in sham treated rats and this effect was reversed by intrathecal morphine

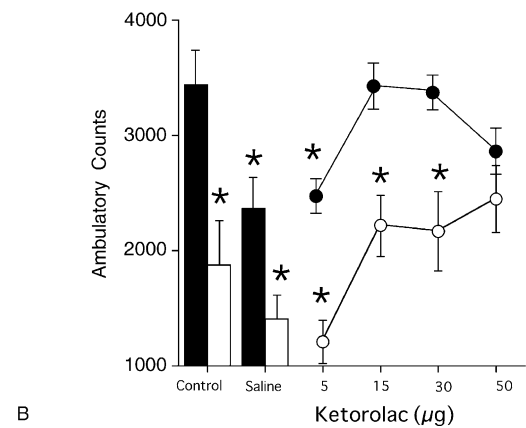
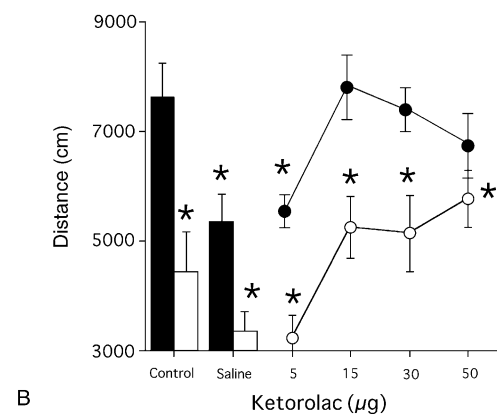
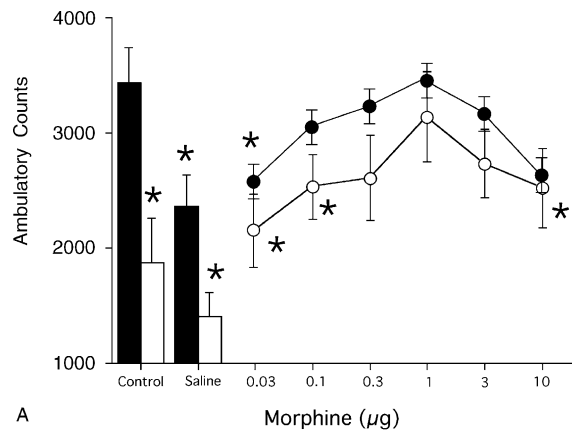
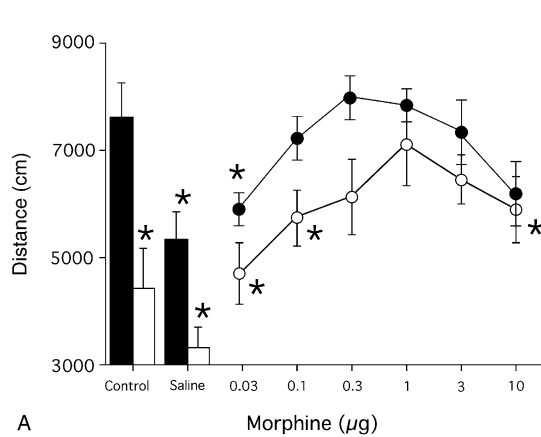
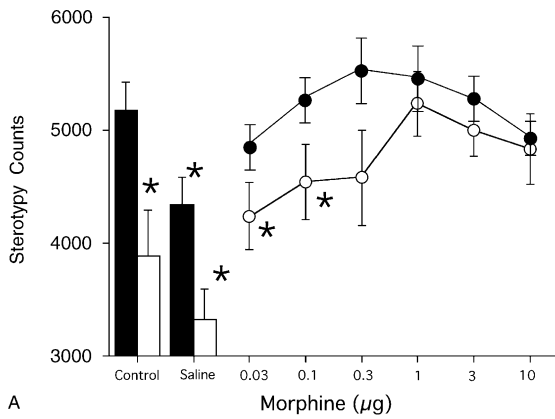


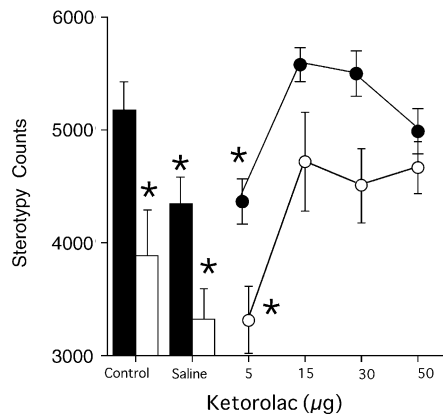
Fig. 3. Distance traveled after abdominal incision following intrathecal injection of morphine (A, top panel) or ketorolac (B, bottom panel). Control rats were not implanted with intrathecal catheters (two leftmost bars). Data from sham-treated rats are indicated by closed bars and circles and from rats subjected to abdominal incision are indicated by open bars and circles. \* $P < 0.05$  compared to sham-treated control.

Fig. 4. Ambulatory counts after abdominal incision following intrathecal injection of morphine (A, top panel) or ketorolac (B, bottom panel). Control rats were not implanted with intrathecal catheters (two leftmost bars). Data from sham-treated rats are indicated by closed bars and circles and from rats subjected to abdominal incision are indicated by open bars and circles. \* $P < 0.05$  compared to sham-treated control.





A



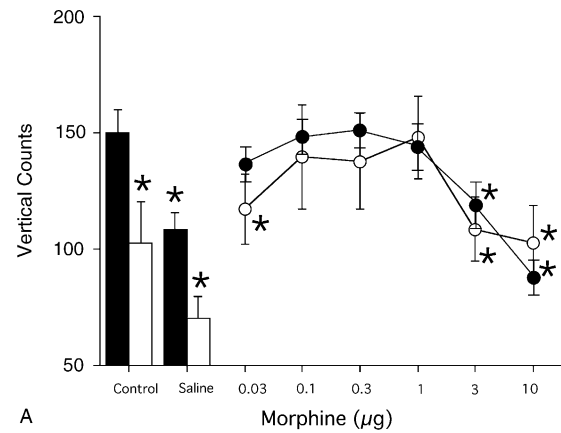
B

Fig. 5. Stereotypy counts after abdominal incision following intrathecal injection of morphine (A, top panel) or ketorolac (B, bottom panel). Control rats were not implanted with intrathecal catheters (two leftmost bars). Data from sham-treated rats are indicated by closed bars and circles and from rats subjected to abdominal incision are indicated by open bars and circles. \* $P < 0.05$  compared to sham-treated control.

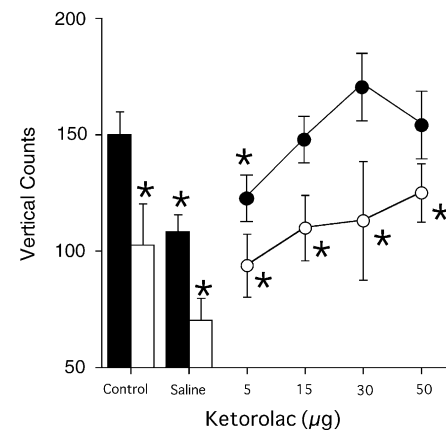
administration. Morphine increased distance traveled [ $F(8,89) = 3.8$ ,  $P = 0.001$ ], ambulation [ $F(8,89) = 3.1$ ,  $P = 0.004$ ], stereotypy [ $F(8,89) = 2.8$ ,  $P = 0.009$ ] and rearing [ $F(8,89) = 3.6$ ,  $P = 0.001$ ]. Doses of morphine at 1 or 3  $\mu\text{g}$  reversed the effect of intrathecal catheterization such that all behaviors were not significantly different following intrathecal administration of these doses compared to those measured in the control sham group ( $P \leq 0.05$ ). The 10  $\mu\text{g}$  dose of morphine significantly decreased all behaviors in sham treated groups relative to the control sham group. Distance traveled, ambulation, stereotypy and rearing was not significantly different between incision and sham groups following administration of 0.3  $\mu\text{g}$  of morphine or higher doses ( $P \leq 0.05$ ).  $E_{\text{max}}$  and  $\text{ED}_{50}$  values are listed in Table 1.

### 3.2.2. Effects of intrathecal ketorolac

Ketorolac, unlike morphine, only partially reversed the effect of abdominal surgery on exploratory behavior in rats. Ketorolac increased the distance traveled [ $F(6,79) = 5.9$ ,  $P < 0.0001$ ], ambulation [ $F(6,79) = 5.5$ ,  $P < 0.0001$ ] and



A



B

Fig. 6. Rearing activity after abdominal incision following intrathecal injection of morphine (A, top panel) or ketorolac (B, bottom panel). Control rats were not implanted with intrathecal catheters (two leftmost bars). Data from sham-treated rats are indicated by closed bars and circles and from rats subjected to abdominal incision are indicated by open bars and circles. \* $P < 0.05$  compared to sham-treated control.

stereotypy [ $F(6,79) = 4.3$ ,  $P = 0.001$ ] in a dose-dependent manner. Post hoc analyses found that only the highest dose of 50  $\mu\text{g}$  of ketorolac significantly increased these three behavioral measures compared to saline administration ( $P \leq 0.05$ ). Distance traveled, ambulation and stereotypy were all significantly less following administration of 50  $\mu\text{g}$  of ketorolac i.t. after abdominal incision compared to the sham control group however ( $P \leq 0.05$ ). Ketorolac did not have any significant effect on rearing at doses up to 50  $\mu\text{g}$  [ $F(6,79) = 2.1$ ,  $P = 0.07$ ]. Ketorolac was able to reverse the effect of intrathecal catheterization on exploratory behavior in the absence of laparotomy [distance traveled  $F(6,72) = 2.4$ ,  $P = 0.04$ ; ambulation  $F(6,72) = 2.3$ ,  $P = 0.05$ ; stereotypy  $F(6,72) = 3.4$ ,  $P = 0.005$ ; rearing  $F(6,72) = 3.1$ ,  $P = 0.01$ ]. In sham-treated animals with intrathecal catheters, ketorolac at doses greater than 5  $\mu\text{g}$  produced a significant increase in all 4 measures of exploratory activity when compared to saline administration and the activity was not significantly different than sham control subjects without intrathecal catheters ( $P \leq 0.05$ ) (Figs. 3–6).  $E_{\text{max}}$  and  $\text{ED}_{50}$  values are listed in Table 1.

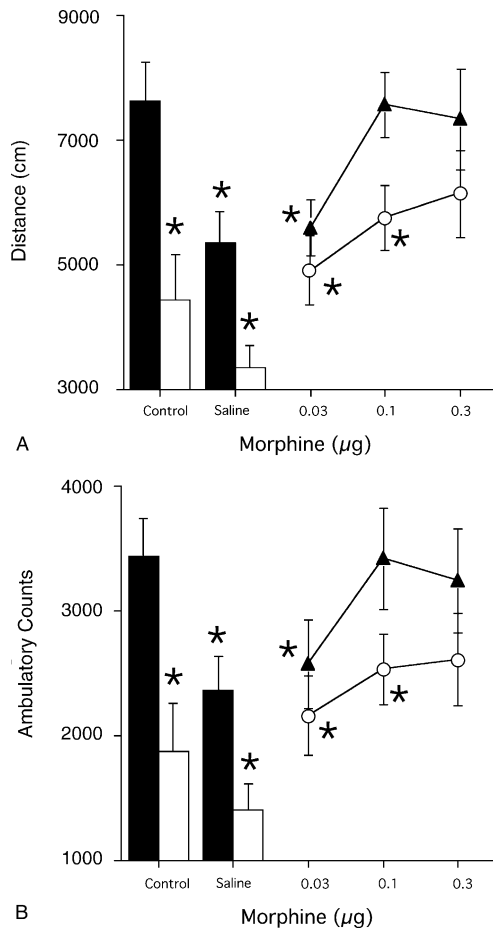


Fig. 7. Distance traveled (A, top panel) and ambulatory counts (B, lower panel) after abdominal incision following intrathecal injection of morphine (open circles) or morphine plus 5 µg of ketorolac (closed triangles). Control rats were not implanted with intrathecal catheters (two leftmost bars). Data from sham-treated rats are indicated by closed bars and from rats subjected to abdominal incision are indicated by open bars. \* $P < 0.05$  compared to sham-treated control.

### 3.2.3. Effects of intrathecal morphine plus ketorolac

Co-administration of 5 µg of ketorolac resulted in a significant increase in the effect of morphine (0.03, 0.1 and 0.3 µg) on exploratory behaviors following laparotomy (Figs. 7 and 8). Administration of 5 µg of ketorolac with morphine did not produce dose-related effects on exploratory behaviors in sham-treated animals [distance traveled  $F(5,59) = 2.3$ ,  $P = 0.06$ ; ambulation  $F(5,59) = 1.8$ ,  $P = 0.13$ ; stereotypy  $F(5,59) = 2.3$ ,  $P = 0.06$ ; rearing  $F(5,59) = 2.1$ ,  $P = 0.08$ ]. Administration of 5 µg of ketorolac with morphine produced dose-related increases in exploratory behaviors following abdominal incision however [distance traveled  $F(5,60) = 6.7$ ,  $P < 0.0001$ ; ambulation  $F(5,60) = 5.6$ ,  $P = 0.0003$ ; stereotypy  $F(5,60) = 6.9$ ,  $P < 0.0001$ ; rearing  $F(5,60) = 8.0$ ,  $P < 0.0001$ ]. Morphine at doses of 0.1 or 0.3 µg in combination with 5 µg of ketorolac significantly elevated all behavioral measures relative to saline administration and all behavioral measures were not significantly

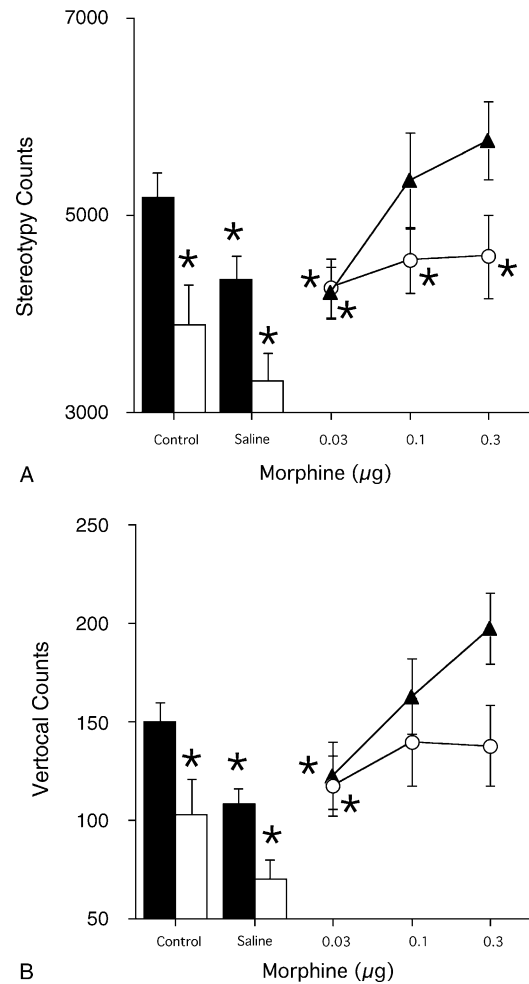


Fig. 8. Stereotypy counts (A, top panel) and rearing counts (B, lower panel) after abdominal incision following intrathecal injection of morphine (open circles) or morphine plus 5 µg of ketorolac (closed triangles). Control rats were not implanted with intrathecal catheters (two leftmost bars). Data from sham-treated rats are indicated by closed bars and from rats subjected to abdominal incision are indicated by open bars. \* $P < 0.05$  compared to sham-treated control.

different from the control sham group ( $P \leq 0.05$ ). The behavior in animals administered 0.1 µg of morphine in combination with 5 µg of ketorolac was significantly greater than that obtained with 0.1 µg of morphine alone for all parameters ( $P \leq 0.05$ ).

## 4. Discussion

Opioids and COX inhibitors are the most commonly used analgesics in the postoperative period, and their effects and interactions have been the subject of numerous clinical and laboratory investigations. Some of the data in the current study are confirmatory, since previous reports indicate that intrathecal morphine (Zahn et al., 1997) and ketorolac (Zhu et al., 2003) reduce tactile hypersensitivity after paw incision, and potentiation of intrathecal morphine by ketorolac is predicted by previous study of this combination

after formalin injection (Malmberg and Yaksh, 1993) and peripheral nerve injury (Lashbrook et al., 1999). Key new information, and the major purpose of the current study, is the comparison of drug effects in two validated models of postoperative pain. Probing with von Frey filaments close to the surgical wound after paw incision or near the territory of an injured peripheral nerve is the most commonly used outcome measure for investigating the biology and analgesic pharmacology of acute and persistent pain states, yet only threshold information is most commonly obtained with this method, and some argue that this withdrawal threshold is not relevant to the chronic pain state (Hogan et al., 2004). The key finding of the current study is that there are qualitative similarities, but quantitative differences between the effects of a COX inhibitor and an opioid after surgery, such that their predictions regarding efficacy and dose in humans differ. Since these drugs are currently being tested in humans for postoperative analgesia (JCE; unpublished data), the predictive value of these two methods to the human condition will soon be measured.

#### 4.1. Maximum efficacy

COX inhibitors alone are indicated for the treatment of mild to moderate pain, whereas opioids are indicated for the treatment of moderate to severe pain, and numerous laboratory and clinical studies under various acute and chronic pain conditions indicate that maximum efficacy of COX inhibitors is less than opioids. It is therefore not surprising that maximum efficacy of intrathecal ketorolac is less than morphine in the current study. Intrathecal morphine was fully efficacious in all outcome measures, including spontaneous rearing activity after laparotomy, a behavior which stretches the skin over the abdominal wound and whose disruption from surgery is not reversed by systemic morphine (Martin et al., 2004). This is consistent with clinical observations of greater analgesia at rest and during movement with intrathecal or epidural than systemic morphine, in doses which do not produce intense sedation in humans, after surgery (Eisenach et al., 1988).

In contrast to morphine, maximum efficacy of intrathecal ketorolac varied depending on outcome measure, with greatest efficacy to block disruption of stereotypy and rearing behaviors, intermediate efficacy to block disruption of ambulatory behavior, and lowest efficacy against von Frey filament testing (Table 1). These data are consistent with a previous study showing that a low dose of aspirin produced antinociception in a complex behavior in an escape avoidance paradigm without altering von Frey testing withdrawal threshold in rats with peripheral inflammation (LaBuda and Fuchs, 2001). These data also predict that intrathecal ketorolac, unlike morphine is unlikely to provide complete analgesia alone in humans after surgery. In general, intrathecal administration of COX inhibitors provides a similar maximum efficacy for antinociception to systemic administration, but with a small fraction of the dose

(Svensson and Yaksh, 2002). In contrast, maximum efficacy from intrathecal injection of ketorolac in the spontaneous behaviors following laparotomy in the current study (30–73%) is considerably greater than the 0–30% observed in the same model with systemic administration (Martin et al., 2004), raising the possibility of greater efficacy in humans with intrathecal than systemic administration.

#### 4.2. Potency

Intrathecal morphine was more potent than ketorolac in all measures after surgery, as expected. Relative potency of morphine to ketorolac varied over 100 fold, depending on outcome measure, with greatest relative potency ratio in favor of morphine for return of disrupted rearing behavior to normal, and least relative potency ratio in favor of morphine for return of disrupted exploratory ambulatory behavior (Table 1). For both drugs, potency was greater for spontaneous behavior than for withdrawal threshold, with a rank order of potency for behaviors rearing > stereotypy > distance > ambulation (Table 1). The ability of lower doses of intrathecal morphine or ketorolac to return rearing behavior to normal relative to the doses required for ambulatory behavior is in contrast to results in this model with systemic morphine, which was not able at any dose to affect rearing behavior (Martin et al., 2004).

As with maximum efficacy, the differences in potency observed for these drugs to withdrawal threshold compared to spontaneous behaviors are qualitatively similar, but provide different quantitative predictions. Assuming a typical ED<sub>50</sub> for clinically relevant analgesia for intrathecal morphine in the 0.2–0.5 mg range, the ratio of potencies to withdrawal threshold observed in the current study predicts an effective intrathecal ketorolac dose range would be 10–25 mg, very similar to the known systemic dose. In contrast, the ratio of potencies to ambulatory behavior in the current study predicts an effective ketorolac dose of only 2–5 mg. Of course, differences in intensity of pain between the two animal models and differences in distribution and penetration of drugs into the larger human spinal cord compared to the rat might confound such predictions, but, at least within the class of opioids, there is a clear and linear relationship between potencies of drugs after intrathecal injection in the rat and their relative potencies in humans (Yaksh et al., 1984).

#### 4.3. Drug interaction

Intrathecal ketorolac enhanced the effect of morphine on spontaneous behavior after laparotomy up to 3.5 fold in the current study, with greater effects on ambulation than on stereotypy and rearing, perhaps due to lack of sensitivity to observe a shift in the latter behaviors, where the lowest doses of morphine produced a 50% or greater effect. These data suggest that intrathecal ketorolac may exert a greater dose-sparing effect on morphine than is observed with systemic



administration, where COX inhibitors reduce opioid consumption after surgery by only 20–30% (Romsing and Moiniche, 2004). The 5 µg dose of ketorolac which potentiated morphine's effects after laparotomy equates on a per kg basis to 1.4 mg in humans, within the dose range being studied (Eisenach et al., 2002). Quantitative examination of the interaction after paw incision indicated a strong synergy, consistent with previous studies of this interaction in other pain models (Kroin et al., 2002; Malmberg and Yaksh, 1993).

#### 4.4. Effect of intrathecal catheter

Chronic intrathecal catheterization of rats, commonplace in analgesia studies since its description nearly 30 years ago (Yaksh and Rudy, 1976), has been reported to alter pharmacologic responses itself in sporadic reports, and produces inflammation observed in histologic studies (Yaksh et al., 1986). The current study demonstrated reduced exploratory activity of rats after intrathecal catheterization, with a greater decrement in these animals after laparotomy than in those without catheterization. Prado previously observed a reduction in intrathecal morphine potency for antinociception to noxious heat to the tail from chronic intrathecal catheterization, and this effect was diminished by indomethacin, suggesting a role for spinal COX (Prado, 2003).

These results raise the possibility that any effect observed from intrathecal ketorolac was merely removing ongoing nociception and its reduction in morphine efficacy as an artifact from the intrathecal catheter itself rather than altering the postoperative pain state. This is unlikely, since we previously demonstrated increases in COX-1 immunoreactivity in the spinal cord ipsilateral to paw incision of non-catheterized rats with a time course similar to that of reduction in withdrawal latency after surgery and its resolution (Zhu et al., 2003).

Finally, although not a subject of the current study, it is important to recognize that another important aspect of pain after surgery is the association between severe postoperative pain and development of chronic pain (Perkins and Kehlet, 2000). At least one clinical trial demonstrated a reduction in the incidence of chronic pain after surgery by addition of ketamine in a dose that had no effect on pain or opioid consumption in the acute recovery period (De Kock et al., 2001). In this regard we note the encouraging recent observation that intrathecal ketorolac, administered for a brief period at the time of surgical nerve injury, permanently prevents the development of hypersensitivity to tactile stimulation (Hefferan et al., 2003).

## 5. Conclusion

In conclusion, intrathecal morphine, ketorolac, and their combination produce qualitatively similar effects on withdrawal response to von Frey filament stimulation after paw

incision and on return to normal spontaneous exploratory behavior after laparotomy. These models differ, however, in prediction of degree of efficacy and potency of intrathecal ketorolac. Intrathecal catheterization itself reduces spontaneous exploratory behavior, and alleviation by intrathecal morphine or ketorolac is consistent with ongoing nociception from this procedure. The powerful enhancement of intrathecal morphine by ketorolac, as well as a previous study indicating that intrathecal ketorolac could reduce the incidence of chronic pain after surgery provide powerful rationales for the study of intrathecal ketorolac alone and with morphine in the postoperative period.

## 6. Summary statement

The relevance of withdrawal threshold from tactile stimulation after surgery in rats to postoperative pain in humans is unclear. Using a recently developed method to assess spontaneous behavior after surgery in rats, we observed similar overall effects of intrathecal morphine, ketorolac, and their combination to antagonize disrupted behavior as compared to withdrawal to tactile stimulation with von Frey filaments, although individual drug potency was 3–5-fold greater to antagonize disrupted behavior than to alleviate hypersensitivity.

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