Effects of Preemptive or Postinjury Intrathecal Local Anesthesia on Persistent Nociceptive Responses in Rats

Confounding Influences of Peripheral Inflammation and the General Anesthetic Regimen

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Background: **Although experimental evidence indicates that preemptive intrathecal treatment with local anesthetics reduces postinjury neuronal hyperexcitability, clinical evidence indicates that preemptive treatments do not consistently reduce postoperative pain. The current study used experimental models of postinjury nociception, in which rats received subcutaneous or intraarticular injections of the irritant formalin, to evaluate the effects of peripheral inflammation, or the use of agents supplemental to anesthesia, as possible confounding influences on the effectiveness of preinjury and postinjury intrathecal local anesthetic treatments.**

Methods: **In experiment 1, lumbar intrathecal lidocaine (30 μl, 2%), given either 5 min before or 5 min after hind paw injection of 50 μl of varying concentrations of formalin, was compared with intrathecal cerebrospinal fluid, for their effects on nociceptive responses in the late phase of the formalin test. Furthermore, the effect of hind paw injection of 50 μl of 2.5, 3.75, or 5.0% formalin on peripheral inflammation was assessed by measuring plasma extravasation in the hind paws of rats given Evans Blue dye (50 mg/kg, intravenous). In experiment 2, rats received a deep tissue injury (100 μl of 5.0% formalin into the knee joint) while under halothane anesthesia. In addition to halothane (3–4%), rats received either saline, pentobarbital (13 mg/kg, intraperitoneal), or pentobarbital + morphine (0.5 mg/kg, intravenous), with or without preinjury or postinjury spinal anesthesia using intrathecal bupivacaine (30 μl, 0.75%), to assess the effects of supplemental treatments on the preemptive effects of intrathecal bupivacaine.**

Results: **Lumbar intrathecal lidocaine pretreatment, but not posttreatment, significantly reduced late phase nociceptive responses to hind paw injections of 2.5% formalin. The preemptive effects of lidocaine were overridden in rats that received hind paw injections of 3.75 and 5.0% formalin. Hind paw injection of 50 μl of 3.75 or 5.0%, but not 2.5% formalin produced an increase in plasma extravasation. Either pentobarbital or pentobarbital + morphine treatment, or a pentobarbital + morphine treatment and postinjury treatment with intrathecal bupivacaine failed to produce a significant reduction in the nociceptive response to the deep tissue injury. However, rats that received pentobarbital + morphine treatments and intrathecal bupivacaine before the injury had significantly reduced nociceptive responses to deep tissue injury when compared to the saline control group, but not to the group that received pentobarbital + morphine treatment and postinjury treatment with bupivacaine.**

Conclusions: **The current results attest to the important effects of ongoing inputs from inflamed tissue, and the use of supplemental treatments, as important confounding factors that may influence the effectiveness of preemptive spinal anesthesia for postoperative pain. (Key Words: Analgesia: preemptive. Anesthesia, local: intrathecal. Pain: postoperative. Spinal cord: nociception. Test: formalin.)**

A growing body of clinical data shows that preoperative local[1–4] or epidural/spinal[4–6] anesthesia, or the

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preoperative systemic administration of analgesic agents[7–10] can significantly reduce postoperative pain or postoperative opioid requirements.[11] The postoperative analgesic effects of such treatments are assumed to depend on the ability of the pretreatment to preempt a sensitization of central nervous system neurons by the injury associated with the surgical intervention; hence, the term preemptive analgesia has been coined for such treatments.[12] While there is considerable evidence demonstrating that peripheral injury, as would occur with surgery, leads to a sensitization of central nervous system neurons,[13–18] the evidence for the ability of preemptive analgesia to attenuate postoperative pain is less convincing.[19,20] Clinical studies comparing preemptive *versus* no treatment were overwhelmingly suggestive of a beneficial effect in pretreated patients, but the value of preemptive treatment became less obvious when compared with the same treatment initiated after surgery. Studies comparing the effectiveness of presurgical *versus* postsurgical treatment with local/regional anesthesia, epidural anesthesia or analgesia, or systemic morphine have produced conflicting results, with some studies indicating a limited[5,10,21,22] or even no[23–26] advantage of presurgical over postsurgical treatment. One explanation for the inability to detect a significant benefit of presurgical administration of these treatments has been that in many clinical trials there is routine use of preoperative or intraoperative opioids as part of the general anesthetic regimen in both presurgical and postsurgical treatment groups.[27,28] Thus, it is possible that the preoperative/intraoperative opioid use may confound the results because they may themselves produce a preemptive effect that reduces postoperative pain. Another explanation is that postoperative pain may depend more heavily on the peripheral inflammation that develops after surgery than on central sensitization occurring during surgery,[11,19,29] and consequently postsurgical treatments are as effective as pretreatments.

In reports of animal studies, the formalin test has been used as a model of injury-induced central sensitization,[30] and as an animal model for studying the potential usefulness of preemptive analgesia.[31–34] Subcutaneous injection of dilute formalin into a rat's paw produces a biphasic response including an early intense response in the first 5 min, and a later moderate response that is expressed from 20 to 60 min after injection.[35] The nociceptive response to formalin is matched by a corresponding biphasic increase in the activity of dorsal horn neurons after such injection.[36] It has been demonstrated that intrathecal administration of either lidocaine[30,32] or opioids[31,36] abolishes behavioral and dorsal horn neuron responses to subcutaneous formalin, if they are administered before but not immediately after the early phase of the formalin response. This suggests that neural activity generated during the early phase of the formalin response is capable of producing changes in central nervous system function, which, in turn influence nociceptive processing during the late phase. The ability of the preinjury treatment with intrathecal lidocaine or opioids to suppress the late phase response to formalin has been described as an animal model of preemptive analgesia, because the pretreatments preempt the central sensitization, which contributes to persistent nociceptive behaviors.

In the current study, we further examined the usefulness of the formalin test as an animal model for assessing preemptive analgesia. In particular, we used the formalin test in rats to examine the effects of both peripheral inflammation and opioid and barbiturate supplements (used routinely as part of a general anesthetic regimen), on the ability to detect differences in postinjury nociceptive responses as a function of preinjury *versus* postinjury treatment with spinal anesthesia (intrathecal lidocaine or bupivacaine). In an effort to bridge the gap between clinical and animal experimental studies, this study was designed to determine the effectiveness of preemptive treatments in animals when both the injury and the anesthetic treatment regimens more closely resemble clinical conditions. Thus, we examined whether increasing peripheral inflammation (by increasing the concentration of formalin injected into the hind paw) reduced the ability of preemptive intrathecal lidocaine treatments to suppress postinjury nociceptive responses. We also examined whether the supplemental treatment with pentobarbital, or pentobarbital + morphine produced a reduction in the ability to detect differences between preinjury *versus* postinjury treatment with intrathecal bupivacaine on the suppression of postinjury nociceptive responses. For this latter purpose, a knee joint injection of formalin was used to produce a condition that more closely resembles postsurgical pain, in which the injury is predominantly in deep rather than cutaneous tissue, and where an initial injury barrage is followed by significant peripheral inflammation, and movement-related pain or hyperalgesia. In addition, bupivacaine was used as the local anesthetic agent, as opposed to lidocaine, to provide a prolonged nociceptive blockade.

Methods and Materials

Animals

The following experiments were carried out under protocols approved by the Institutional Animal Care Committee of the Clinical Research Institute of Montreal. Male Long Evans hooded rats (weighing 275–375 g) were used in these studies. The rats were housed individually (catheterized rats) or in groups of 3 or 4 (no catheters), and had access to food and water at all times.

Intrathecal Catheters

In animals that received intrathecal lidocaine, bupivacaine, artificial cerebrospinal fluid (CSF) or saline, chronic lumbar intrathecal catheters were implanted while rats were anesthetized with 65 mg/kg intraperitoneal sodium pentobarbital (Somnotol, MTC Pharmaceuticals, Cambridge, Ontario, Canada). The catheter (Intramedic PE-10 tubing, Clay Adams, Parsippany, NJ) was inserted through an incision in the dura mater at the atlantooccipital junction, and was positioned so that the inner end of the catheter lay at the lower lumbar (L4–L6) spinal level. The outer end of the catheter was fixed with dental cement to a screw embedded in the skull. The rats were allowed to recover for 4–6 days and only those animals that were free of any neurologic deficit were used in the experiments. The location of the inner end of the catheter was verified during postmortem examination.

Formalin Test

Formalin-induced nociceptive behaviors were measured in rats that received an injection of 50 μl of either 2.5, 3.75, or 5.0% formalin into the plantar surface of one hind paw. For nociceptive testing, each rat was placed in a 30 cm × 30 cm × 30 cm methyl methacrylate polymer box with a mirror below the floor at a 45° angle to allow an unobstructed view of the paws. A nociceptive score was determined using the weighted scores method of behavioral rating devised by Dubuisson and Dennis.[35] Briefly, this involved the measurement of the time spent in each of four behavioral categories: (0) the injected paw is not favored, (1) the injected paw has little or no weight on it, (2) the injected paw is elevated and is not in contact with any surface, and (3) the injected paw is licked, bitten, or shaken. A weighted average nociceptive score, ranging from 0 to 3, was calculated by multiplying the time spent in each category by the category weight, summing these products, and then dividing by the total time of the test. Concentrations of formalin ranging from 2.5 to 5.0% are routinely used in the formalin test, as is the 50-μl volume.

Plasma Extravasation

The degree of peripheral inflammation produced by various concentrations of formalin was assessed by measuring plasma extravasation in the hind paw of untreated rats and rats injured by subcutaneous injection of saline or 2.5, 3.75, and 5.0% formalin into the plantar surface of the hind paw. To measure plasma extravasation, rats were given an intravenous (tail vein) injection of Evans Blue dye (50 mg/kg in 2.5 ml/kg) 30 min before the hind paw injury. Rats were then killed 45 min after the formalin injection by overdose of 200 mg/kg intraperitoneal sodium pentobarbital. After intracardiac perfusion with 0.9% saline to flush blood from the circulation, the hind paws of untreated and injured rats were removed by amputation at the ankle joint. The hind paws were then incubated in 4 ml formamide at 70°C for 24 h to extract Evans blue dye from the tissue. After cooling to room temperature, plasma extravasation was recorded as the absorbance of the resulting supernatant in a spectrophotometer at a wavelength of 620 nm.

Knee Joint Injury

Rats were given an injection of 100 μl 5.0% formalin into the knee joint while anesthetized with 3% halothane. Preliminary investigations demonstrated that this volume of 5.0% formalin produced flinching responses in lightly anesthetized rats for about 60–70 min, followed by a persistent inflammation in the rat knee joint during a prolonged postinjury period (2–3 days). To approximate the events during surgery in which patients are anesthetized when the most intense tissue injury and afferent barrage occurs, rats were deeply anesthetized with halothane before and for 45 min after the knee injury. Rats typically recovered from the halothane anesthesia between 25 and 30 min after its termination (*i.e.*, 70–75 min postformalin).

Nociceptive assessment began 30 min after termination of the halothane anesthesia (*i.e.*, 75 min postformalin), and was additionally performed at 1, 2, 4, 24, and 48 h. Nociception during this postinjury inflammatory period is relatively static over prolonged periods, and thus a nociceptive score can be generated by observing the rats and recording their behaviors over a brief (5 min) time period at each time point. Noci-

ceptive behaviors during the postinjury inflammatory period were assessed on a 5-point behavioral scale, which was defined as follows: 0 = equal weight on both hind paws; 1 = paw is completely on the floor but the toes are not spread; 2 = foot is curled with only some parts of the foot lightly touching the floor; 3 = foot is completely elevated; 4 = the rat licks the injured knee or any other part of the hind limb. The experimenter, who was unaware of the treatment condition, recorded the highest nociceptive behavior observed during a 5-min period at the given observation time.

Procedure

Experiment 1. Assessment of the Effect of Intrathecal Lidocaine on Nociceptive Responses to Different Concentrations of Formalin. The first experiment examined the influence of peripheral inflammation on the ability of spinal anesthesia to preempt nociceptive behaviors in the formalin test in a total of 12 groups of rats. Formalin-induced nociceptive responses were measured in six groups of rats that received intrathecal lidocaine either 5 min before or 5 min after a subcutaneous injection of 50 μl of either 2.5, 3.75, or 5.0% (preformalin and postformalin groups) formalin into the plantar surface of one hind paw. Spinal anesthesia was produced by administering a large bolus (30 μl) of 2.0% lidocaine through the chronic intrathecal catheter, followed by 10 μl artificial CSF (an aqueous solution of 128.6 mM NaCl, 2.6 mM KCl, 1.0 mM MgC12, and 1.4 mM CaCl2, phosphate buffered to *p*H 7.33) to flush the catheter, while rats were briefly hand-held. Preliminary experiments indicated that spinal lidocaine produces a complete anesthetic blockade between 2 and 7 min, with partial effects lasting until 20 min posttreatment. Another six groups of rats received CSF (30 μl, intrathecal) 5 min before or after subcutaneous injection of 2.5, 3.75, or 5.0% formalin. Nociceptive testing was performed between 30 and 60 min after formalin injection during the stable tonic late phase of the formalin test. The goal was to assess the limits of the preemptive effects of spinal anesthesia with increasing degrees of peripheral inflammation produced by higher concentrations of formalin.

Further studies were performed to confirm that higher concentrations of formalin do indeed produce a greater degree of peripheral inflammation. Thus, additional rats (n = 4/group) were assessed for the degree of plasma extravasation within the hind paw after subcutaneous administration of either saline, 2.5, 3.75, or 5.0% formalin. The injected hind paw was removed and assessed for Evans Blue dye leakage 45 min after injection of formalin or saline. This time corresponds to the halfway point in the testing of rats in experiment 1. The plasma extravasation produced by the three concentrations of formalin, or by saline, was compared with baseline plasma extravasation in uninjected hind paws of control rats.

Experiment 2. Assessment of the Interactive Effects of Opioid and Barbiturate Supplements with Preinjury and Postinjury Spinal Blocks on Postinjury Nociceptive Responses. A second experiment was performed to examine the influence of opioid and barbiturate supplements on the ability to detect differences in preinjury and postinjury treatments with intrathecal bupivacaine in an animal model of postoperative pain that involved injury of deep tissue in the rat knee joint. Rats were randomly assigned to one of five treatment groups that received combinations of the treatments pentobarbital (13 mg/kg intraperitoneal) and morphine (0.5 mg/kg intravenous), followed by preinjury or postinjury treatment with bupivacaine (30 μl 0.5% intrathecal), or appropriate vehicle control treatments, in addition to general inhalation anesthesia with 2.0–3.0% halothane. The 0.5-mg/kg dose of morphine was chosen because this dose has been found to inhibit the sensitization of the dorsal horn neurons in response to electrical activation of C-fibers.[37] The 13-mg/kg dose of pentobarbital was chosen to produce sedation; although subanesthetic, this dose is higher than the levels of pentobarbital that have been found to produce hyperalgesia, or reverse the antinociceptive effects of morphine in rats.[38–40] Preliminary experiments indicated that spinal bupivacaine produces a complete anesthetic blockade between 5 and 25 min, with partial effects lasting until 45 min posttreatment. The procedure was designed so that the rats received: (1) intraperitoneal pentobarbital or saline ~45 min before the injury; (2) halothane inhalation beginning ~35 min before injury and continuing until 45 min after injury; (3) intravenous (tail vein injection) morphine or saline ~25 min before injury; (4) intrathecal bupivacaine 5 min before injury and intrathecal saline 5 min after injury (pretreatment group) or intrathecal saline 5 min before and intrathecal bupivacaine 5 min after injury (posttreatment group); and (5) a knee joint injection of 100 μl 5.0% formalin. Specifically, the five treatment groups received intraperitoneal/intravenous/intrathecal/intrathecal injections of either: (1) saline/

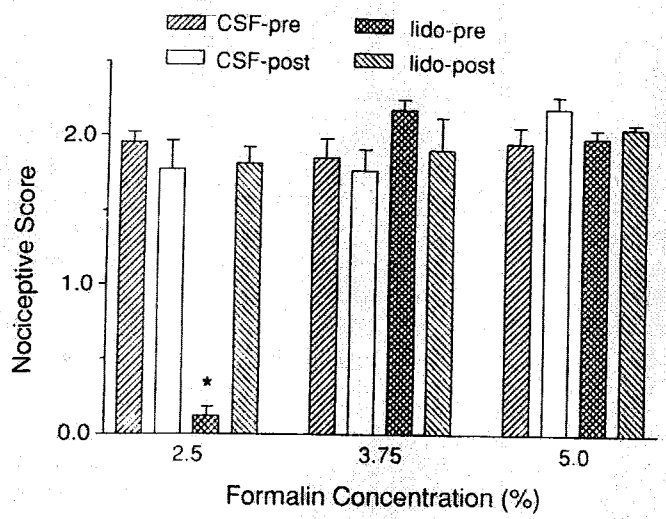

Fig. 1. Nociceptive scores (±SEM) in response to a 50-μl hind paw injection of formalin in rats given intrathecal cerebrospinal fluid or lidocaine (30 μl, 2.0%) 5 min before (CSF-pre, n = 8,8,7 or lido-pre, n = 9,12,10 for 2.5, 3.75 and 5.0% formalin, respectively) or 5 min after (CSF, n = 6,6,6 or lido-post, n = 10,8,6 for 2.5, 3.75 and 5.0% formalin, respectively) the formalin injection. Analysis of variance reveals a significant effect of treatment ($F(3,77) = 22.6$, $P < 0.001$) and formalin concentration ($F(2,77) = 47.1$, $P < 0.001$), as well as a significant treatment X formalin concentration interaction ($F(6,77) = 27.9$, $P < 0.001$). Significant differences from the cerebrospinal fluid pre and post group for each concentration are indicated by an asterisk (*$P < 0.05$, Newman Keuls).

saline/saline/saline; (2) pentobarbital/saline/saline/saline; (3) pentobarbital/morphine/saline/saline; (4) pentobarbital/morphine/bupivacaine/saline; or (5) pentobarbital/morphine/saline/bupivacaine.

Data Analyses

Nociceptive scores for each rat in experiment 1 were averaged over the 30-min test period and compared across groups using one-way analysis of variance. Multiple comparisons were performed using Newman Keuls tests. Measures of plasma extravasation were compared across groups using one-way analysis of variance, followed by Newman Keuls comparisons. Nociceptive scores in experiment 2, were compared across groups at each time point using Kruskal Wallis analysis of ranks, followed by Mann Whitney *U* tests.

Results

Experiment 1. Effect of Intrathecal Lidocaine on Nociceptive Responses to Different Concentrations of Formalin. Figure 1 illustrates the effects of increasing concentrations of formalin in rats given CSF or lidocaine intrathecally before or after formalin treatment. For rats pretreated or posttreated with CSF, there was no significant change in nociceptive responses to formalin as the concentration was increased from 2.5 to 5.0% ($P > 0.05$). Similarly, there was no significant effect of increasing the concentration of formalin in rats given lidocaine 5 min after formalin ($P > 0.05$). When rats were given lidocaine 5 min before formalin, nociceptive responses to 2.5% formalin were significantly decreased compared with rats pretreated with CSF ($P < 0.05$). When the concentration of formalin was increased to 3.75 or 5.0%, there was a reduction in the ability of the preinjury lidocaine treatment to reduce nociceptive responses. In rats pretreated with lidocaine, the nociceptive responses to 3.75 and 5.0% formalin were significantly greater than the responses to 2.5% formalin ($P < 0.05$).

Figure 2 illustrates the degree of plasma extravasation in the hind paws of untreated rats and rats given a hind paw injection of saline or 2.5, 3.75, or 5.0% formalin. Plasma extravasation was reflected by the spectrophotometric measurement of the absorbance at 620 nm of the Evans blue dye extracted from the hind paw. Untreated control rats had a mean (±SEM) absorbance

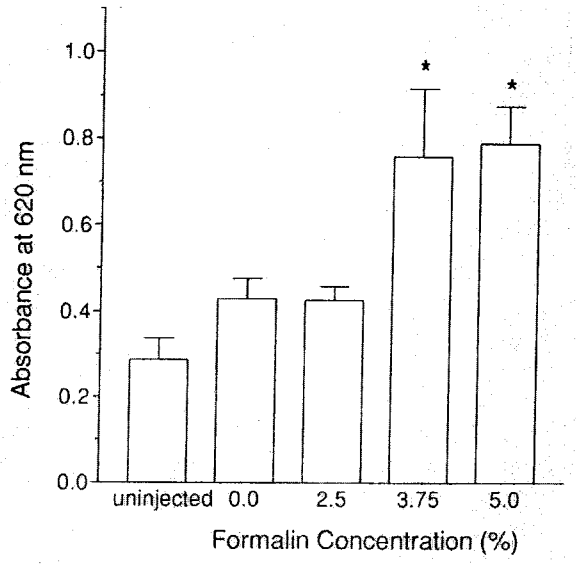

Fig. 2. Plasma extravasation measured as absorbance of Evans Blue dye at 620 nm from one hind paw of uninjected control rats, and in rats with a hind paw injection of 50 μl of either saline (0.0% formalin), or 2.5, 3.75, or 5.0% formalin (n = 4 for each group). Analysis of variance reveals a significant effect of treatment ($F(4,15) = 6.6$, $P < 0.01$). Significant differences from the saline control group are indicated by asterisks (*$P < 0.05$, Newman Keuls).

measurement of 0.29 ± 0.05 at 620 nm. Hind paw plasma extravasation was not significantly affected, as compared with untreated control rats, in rats given a hind paw injection of either saline (absorbance at 620 nm = 0.43 ± 0.05) or 2.5% formalin (absorbance at 620 nm = 0.43 ± 0.03; $P > 0.05$). In contrast, hind paw plasma extravasation was significantly increased, as compared with untreated control rats and also saline-treated rats, in rats given 3.75 or 5.0% formalin (absorbance at 620 nm = 0.76 ± 0.16 for 3.75% formalin, and 0.79 ± 0.09 for 5.0% formalin; $P < 0.05$).

Experiment 2. Interactive Effects of Opioid and Barbiturate Supplements with Preinjury and Postinjury Spinal Blocks on Postinjury Nociceptive Responses. Figure 3 illustrates the nociceptive responses to knee injection of formalin in rats given intraperitoneal/intravenous/intrathecal/intrathecal treatments with: (1) saline/saline/saline/saline, (2) pentobarbital/saline/saline/saline, (3) pentobarbital/morphine/saline/saline, (4) pentobarbital/morphine/bupivacaine/saline (preinjury bupivacaine group), or (5) pentobarbital/morphine/saline/bupivacaine (postinjury bupivacaine group). Control rats given only saline treatments had relatively high nociceptive scores that decreased gradually over the 48 h of measurement from a high of 3.6 ± 0.2 at 30 min to a low of 2.5 ± 0.4 at 48 h. Although rats receiving pentobarbital or pentobarbital + morphine supplemental treatments had nociceptive scores that were consistently lower than those of control rats, these differences were not statistically significant ($P > 0.05$). Rats that received both pentobarbital + morphine supplements and bupivacaine treatment after the injury (bupivacaine posttreatment) also did not differ significantly from the control group ($P > 0.05$). In fact, the only group that exhibited nociceptive scores that were significantly lower than the control group was the group that received both pentobarbital + morphine supplements and bupivacaine treatment before the injury (bupivacaine pretreatment; $P < 0.05$). The significantly lower nociceptive scores in the bupivacaine pretreatment group are attributed to the significantly lower nociceptive scores at 24 and 48 h after injury ($P < 0.05$), where scores ranged from 1.5 ± 0.21 to 1.0 ± 0.26, as opposed to 2.62 ± 0.37 to 2.5 ± 0.42 for the control group during the same period. Importantly, although the bupivacaine pretreatment group had nociceptive scores that were significantly lower than the control group, their scores were not significantly different from the nociceptive scores of rats in the bupivacaine posttreatment group ($P > 0.05$). At the same time, the posttreatment bupivacaine group did not differ significant from any of the other groups, including the control group or the groups that received pentobarbital or pentobarbital + morphine supplements ($P > 0.05$).

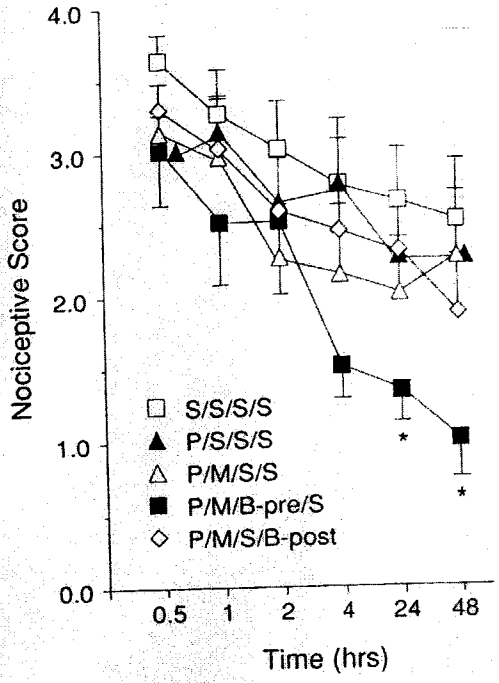

Fig. 3. Time course of the nociceptive scores (± SEM) of rats given a knee joint injection of 100 μl 5.0% formalin and treated with intraperitoneal/intravenous/intrathecal/intrathecal injections of (1) saline/saline/saline/saline (S/S/S/S, n = 8), (2) pentobarbital/saline/saline/saline (P/S/S/S, n = 8), (3) pentobarbital/morphine/saline/saline (P/M/S/S, n = 8), (4) pentobarbital/morphine/bupivacaine/saline (P/M/B-pre/S, n = 6), or (5) pentobarbital/morphine/saline/bupivacaine (P/M/S/B-post, n = 7), in addition to halothane inhalation anesthesia extending from 35 min before injury until 45 min after injury. All treatments were pretreatments except the final intrathecal injection which followed the formalin injection (see Methods). Time is measured from the cessation of the halothane anesthesia which was 45 min after the formalin injection. Significant differences from the S/S/S/S control group are indicated by asterisks (*$P < 0.05$, Mann Whitney U test).

Discussion

The current study examined, in experimental models in rats, two critical factors that have been proposed to influence the effectiveness of preemptive analgesia. The first factor we considered was whether continued afferent inputs driven by inflammatory processes in the injured tissue override the contribution of a sensitization of central neurons to postoperative pain in humans, or in this case postinjury nociception in animals.

The second factor we considered was whether barbiturate or opioid supplements, used as part of the general anesthetic regimen, reduces the ability to obtain differences in postoperative pain or postinjury nociception that occur when comparing preoperative and postoperative/injury treatment with spinal anesthetics. Experiments were also included to determine the degree of inflammation (plasma extravasation) produced by hind paw injections of varying concentrations of formalin.

The results of the first experiment demonstrated that the ability of intrathecal lidocaine to preempt postinjury nociception in the formalin test was lost as the concentration of formalin was increased from 2.5 to either 3.75 or 5.0%. A strong preemptive effect (*i.e.*, a significant reduction in nociceptive scores) of spinal lidocaine was obtained in rats given 2.5% formalin. This preemptive effect was lost (resulting in significantly higher nociceptive scores) in rats given 3.75 and 5.0% formalin. Furthermore, it was shown that injection of 3.75 and 5.0% formalin produced a significant degree of inflammation (plasma extravasation). In contrast, the degree of inflammation produced by 2.5% formalin was not significantly different from that produced by the same volume of saline, and was only slightly, but not significantly higher than no injection at all. Thus, the preemptive effects of intrathecal lidocaine are greatest when there is little or no inflammation, and they decrease directly with increases in peripheral inflammation. These results suggest that it may be difficult to demonstrate a significant effect of preemptive analgesia in patients undergoing major surgery, which is accompanied by considerable local inflammatory changes. The peripheral inflammatory changes and afferent input associated with postoperative inflammation may progressively override the beneficial effects of blocking the afferent barrage at the time of surgery.

Our data on the degree of inflammation or plasma extravasation produced by formalin injection is consistent with, and also extends, our current knowledge in this area. It has been previously demonstrated that injection of between 4.0 and 5.0% formalin into a rat's hind paw produces an increase in paw volume of approximately 30–35% 1 h after injection.[41,42] Furthermore, injection of 5.0% formalin into a single rat toe produces about a 235% increase in plasma extravasation within the skin of the injected toe, over that of an injection of an H_2O vehicle.[43] The current data indicate that after 45 min, injections of either 3.75 or 5.0% formalin produce an approximate 175–185% increase in plasma extravasation in the rat hind paw over that of an injection of a saline vehicle. In contrast, there was no difference whatsoever in plasma extravasation produced by injection of 2.5% formalin (the concentration for which preemptive spinal lidocaine was most effective) as compared with a control injection of saline (absorbance at 620 nm was 0.43 for both groups). It should be pointed out that a concentration of 2.5%, formalin is commonly used in the formalin test, and produces a high degree of nociceptive responses that follow the typical persistent and biphasic time course seen with higher concentrations of formalin. Although saline produced a similar degree of plasma extravasation as 2.5% formalin, a saline injection produces minimal nociceptive responses, which for most rats last only 1–2 min after injection.[44,45] Thus, it appears that low concentrations of formalin (2.5% or less) are capable of producing persistent nociceptive behaviors that are both largely independent of extensive peripheral inflammation (fig. 2), and are highly susceptible to the preemptive effects of spinal lidocaine (fig. 1). On one hand, high concentrations of formalin (3.75% or higher) also produce persistent nociceptive behaviors, but on the other hand, they are associated with significant peripheral inflammation (fig. 2), and are less susceptible to the preemptive effects of spinal lidocaine (fig. 1). It could be argued that the loss of a preemptive effect with higher concentrations of formalin is due to a breakthrough of a larger afferent input through the anesthetic blockade. We believe this is unlikely because we have found that the same dose of intrathecal lidocaine used in the current study produces a complete block of a pressor response to hind paw injection of 10% formalin, without affecting increases in mean arterial pressure in response to forepaw injection of 10% formalin.§

The suggestion that responses to high concentrations of formalin depend more on peripheral inflammation than central sensitization may explain the recent finding that there is no difference between pretreatment and posttreatment with intrathecal lidocaine‖ or excitatory

§ Yashpal K, Coderre TJ, Henry JL: Effects of intrathecal lidocaine on noxious stimulus-induced changes in cardiovascular function, 1995, manuscript in preparation.

‖ Chapman V, Dickenson AH: The effect of intrathecal pre and post-treatment of lignocaine or CNQX on the formalin response of rat dorsal horn neurons. Abstracts of the 7th World Congress on Pain, Seattle, IASP Publications. 1993, p 469

amino acid[46] antagonists on the dorsal horn neuronal responses to a peripheral injection of 5.0% formalin to rats' toes, and why posttreatment with the NMDA antagonist AP5 produced a significant reduction in nociceptive responses to hind paw injection of 10% formalin in mice.[47] Furthermore, late phase dorsal horn neuronal responses to 5.0% formalin are significantly reduced by local anesthesia of the injected area at the time of testing,[48] but not by a prior local anesthesia of the injected area during the early phase.[49] This is consistent with the hypothesis that the persistent (late phase) nociception produced by a higher concentration of formalin is much more dependent on the ongoing afferent barrage associated with significant peripheral inflammation during the late phase. The mechanisms responsible for generating nociceptive behavior after administration of a low concentration of formalin differ markedly from the mechanisms present after a high concentration of formalin. Thus, it is important to pay careful attention to the concentration of formalin that the investigators have used, when comparing the results of different studies using the formalin test.

The second experiment examined whether administration of a barbiturate (pentobarbital), or a barbiturate and an opioid (morphine) as part of the general anesthetic regimen, produced a reduction in the ability to detect differences between preinjury and postinjury treatment with intrathecal bupivacaine on the suppression of postinjury nociceptive responses. Groups of rats were compared on postinjury nociceptive scores after saline treatment, barbiturate or barbiturate and opioid supplements, or barbiturate and opioid supplements with either preinjury or postinjury treatment with intrathecal bupivacaine. Results in this experiment revealed that only the rats pretreated with intrathecal bupivacaine had significantly lower nociceptive scores than those in the saline control group. Surprisingly, this significant reduction was restricted to the latter observations at 24 and 48 h. We speculate, based on our results in the first experiment, that the overall background nociceptive level (associated with peripheral inflammation) must fall below a critical level before the effects of the preemptive treatment become obvious.

It should be noted that the lack of significant differences among the treatment groups including supplemental treatment with pentobarbital, pentobarbital and morphine, or the intrathecal bupivacaine posttreatment may have been due to the relatively small group sizes used in the current experiment. While the bupivacaine pretreatment group had significantly lower nociceptive scores than the saline control group, it did not differ significantly from the bupivacaine posttreatment group. We speculate that while the pentobarbital and morphine supplements did not produce a great enough reduction in nociceptive scores to make them significantly lower than those of control rats, these agents may have lowered nociceptive scores just enough to preclude significant differences between the nociceptive scores of rats given pretreatment *versus* posttreatment with bupivacaine. Similarly, barbiturates and opioids, which are administered as supplements to most standard general anesthetic regimens, may create subtle preemptive effects that reduce postoperative pain to a level that could make it difficult to distinguish a beneficial effect of anesthetic or opioid pretreatment over that of the same agent given as a posttreatment.

It has been argued[50] that the discrepancy between the results of animal studies of central sensitization and clinical trials of preemptive analgesia, implies that clinical advances can only be made through the study of pain mechanisms in humans. We believe the current experimental approach and findings dispel this notion, and provide evidence that bridges the gap between animal and human investigations of preemptive analgesia. The results of the current study point to key differences between the animal and clinical literature that may explain some of the discrepancies between the effectiveness of preemptive treatments for reducing central sensitization and for alleviating postoperative pain. It has recently been pointed out[20] that human clinical studies often are plagued with sometimes unavoidable and sometimes avoidable design flaws including (1) the failure to compare pretreatment groups with a group receiving the same agent as a posttreatment (as opposed to a no treatment control group),[1-4,6-9] (2) the use of preoperative or intraoperative opioids in all groups,[2,23-25] (3) the potential preemptive effect of the local anesthetic used to test the position of the epidural catheter,[22,23] and (4) the failure to test the effectiveness of the anesthetic block.[2,24,25] Furthermore, as previously suggested,[19,23,29] there is a tendency to overlook the importance of the contribution to postoperative pain of ongoing inputs from damaged peripheral tissue, and the local inflammatory changes that continue for days after the surgery has been completed. The current results clearly demonstrate the critical role of peripheral inflammation in obscuring the preemptive analgesic effects of spinal lidocaine on postinjury nociception. By comparing groups of rats receiving no treat-

ment or preinjury *versus* postinjury spinal anesthetic blocks (with and without supplemental barbiturate and opioid treatments), the current results also provide experimental evidence that the use of barbiturate and opioid supplemental treatments may obscure the ability to detect differences between preinjury and postinjury (or preemptive *vs.* postsurgical) treatments on postinjury nociception (or postoperative pain).

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