Interaction of Intrathecal Morphine with Bupivacaine and Lidocaine in the Rat

John P. Penning, M.D., F.R.C.P.C.,* Tony L. Yaksh, Ph.D.†

The interaction in the rat between intrathecal morphine and local anesthetics (bupivacaine and lidocaine) on nociception (52.5°C hot plate and paw pressure), motor function, and autonomic function (blood pressure [BP] and heart rate [HR]) was examined over a range of doses for both morphine and the local anesthetics. High doses of intrathecal bupivacaine (75 μg) or lidocaine (500 μg) produced motor block and hypotension (150 μg bupivacaine) lasting approximately 15 and 7 min, respectively, whereas low doses of intrathecal bupivacaine (25 μg) and lidocaine (100 μg) produced only a transient motor weakness lasting 2 min or less. Alone, neither agent altered the hot plate or paw pressure response at doses, or at times, where the agents had no effect upon motor function. In contrast, at the low dose of either local anesthetic, after the resolution of the transient motor weakness, these doses resulted in a significant leftward shift in the dose-response curves for intrathecal morphine on both the hot plate and paw pressure, as measured by the maximum observed peak effect and by the area under the time-effect curve. Thus, for example, the morphine ED50 (95% confidence intervals) for morphine/saline was 1.7 μg (0.7–1.9) on the hot plate and 1.1 μg (0.8–1.4) on the paw pressure versus for morphine/bupivacaine (25 μg): hot plate 0.25 μg (0.21–0.42) and paw pressure 0.28 μg (0.2–0.4). Intrathecal morphine was not observed to have any effect on the dose-dependent effects of intrathecal bupivacaine on motor or autonomic blockade. Comparable results were also observed with lidocaine (bupivacaine was found to have no significant effect on spinal cord morphine clearance). We conclude that low doses of intrathecal lidocaine and bupivacaine, which alone have no antinoceptive effect, at times when motor function was clearly unimpaired, are able to significantly augment the antinoceptive activity of intrathecal morphine on the hot plate and paw pressure tests. This prominent and selective potentiation appears to occur via a non-pharmacokinetetic mechanism and probably reflects upon the interaction of low concentrations of local anesthetics with systems in the spinal dorsal horn that process acute high threshold afferent input. (Key words: Analgesics, opioid: morphine. Anesthetic techniques: spinal. Anesthetics, local: bupivacaine; lidocaine. Pain: antinoception.)

THE EPIDURAL OR intrathecal administration of morphine is a highly efficacious modality for the treatment of acute pain.1,2 However, side effects are often present (e.g., sedation, pruritus, and urinary retention) and rarely are life-threatening (respiratory depression).3,4 In an attempt to diminish side effects, yet maintain or enhance analgesic efficacy, clinicians have combined local anesthetics and epidural morphine with apparent good results.5–7

Extensive support exists in the clinical literature that the addition of opioids significantly augments the analgesic efficacy of epidurally administered local anesthetics.8–15 However, there are few clinical studies that have attempted to define systematically the analgesic effects of the joint spinal administration of local anesthetics and opioids. Some investigators have reported enhancement of analgesia, while others report no evidence of any additive effect.16–18 The particular significance of the interaction is that significant synergy would diminish the amount of opioid required to attain the desired degree of analgesia and thereby diminish distribution-related opioid side effects. Such advantages of local anesthetic would, however, be accordingly diminished if augmentation was observed only at doses that produced clinically significant somatomotor or autonomic block, or if the combined effects also facilitated the autonomic and motor effects of the local anesthetic.

Considering the clinical importance of this issue, there is a paucity of basic animal work evaluating the spinal interaction between local anesthetics and opioids. Large doses of bupivacaine and lidocaine potentiate spinal morphine antinoception in a synergistic fashion in the hot plate and tail-flick tests in mice19,20 and rats,21,22 but the changes in motor and autonomic function were not assessed. In recent studies, Maves and Gebhart21 have described antinoceptive synergy between (intrathecal) morphine and lidocaine using several nociceptive endpoints. Those investigators examined the effects of drugs at time of peak anesthetic action and perhaps not surprisingly observed a significant nonlinear interaction. An important consideration is the question of whether the effect upon a motor response is contributing to the observed behavioral response. In our work, preliminary studies revealed the surprising observation that even following the resolution of all detectable motor deficit, there was powerful potentiation of the clear analgesic action of spinal morphine, without any augmentation of the effects of morphine on the motor or autonomic effects of the spinal anesthetic. We thus sought in the present experiments to evaluate systematically the interaction in the rat.
between intrathecal morphone and local anesthetics (bupivacaine and lidocaine) on nociception (52.5° C hot plate and paw pressure), while systematic experiments evaluating the motor function and autonomic function (blood pressure [BP] and heart rate [HR]) were performed with intrathecal morphine and bupivacaine. In addition, as drug interactions may reflect changes in drug distribution, the effects of bupivacaine on spinal morphine levels were examined.

**Methods and Materials**

**ANIMAL MODEL**

All experimental procedures were approved by the institutional review board for animal research. Male Sprague-Dawley rats (275–375 g; Harlan Sprague-Dawley, IN) were housed in individual cages with free access to food and water and maintained on a 12-h light-dark cycle at an ambient temperature of 20–23°C. Chronic intrathecal catheters were implanted under halothane anesthesia as previously described. Briefly, through an incision in the atlanto-occipital membrane, a polyethylene (PE-10) catheter, filled with 0.9% saline, was advanced 9 cm caudally to position its tip at the level of the lumbar enlargement. The rostral tip of the catheter was passed subcutaneously, externalized on top of the skull, and sealed with a stainless steel plug. The wound was closed with silk sutures. Rats were not used sooner than 4 days nor later than 21 days after implantation. A maximum of three experiments were conducted on each rat with a minimum of 3 days between studies. Only animals with normal motor function (gait, left and right righting reflexes, and placing and stepping reflexes) and normal baseline responses in the paw pressure and the hot plate tests were used.

**NOICEPTIVE TESTS**

**Hot Plate**

Animals were placed unrestrained on a flat stainless steel surface measuring 25 × 25 cm, maintained at 52.5°C, and enclosed by plexiglass on the sides. The behavioral endpoint was licking of one hindpaw or jumping with both hind feet off the plate. Cut-off time in the absence of a response was 60 s.

**Paw Pressure**

A commercially available device (manufactured by Ugo Basile, Milan) was used. The head and body of the rat was loosely wrapped in a soft cloth and gently held in the left hand of the observer. The hind paw was then placed on a flat plastic surface and pressure was applied to the unrestrained dorsal surface of the hind paw by a small conical projection with a rounded tip measuring 2 mm across. The tip was placed over the middle of the third metatarsal. The device is capable of progressively increasing the pressure on the rat's foot by moving a weight along a horizontal beam. Thereby, the equivalent weight directly over the conical projection was increased uniformly at a rate of 40 g/s until a withdrawal response was observed or the cut-off point of 400 g was reached.

Rats were acclimated to the procedures on the day prior to the experiment and only rats with normal baseline responses were used (hot plate 12–20 s; paw pressure 80–120 g). Time of testing varied with the specific experimental protocol (see below).

**MOTOR FUNCTION**

To achieve a sensitive numeric index, motor function was assessed by bilaterally grading the following: 1) the righting reflex, 2) the placing/stepping reflex, and 3 and 4) the muscle tone of the upper and lower limbs (0 = absent, 1 = impaired, 2 = normal), the normal baseline aggregate score being 8 × 2 = 16. Muscle tone was graded as absent when the limb was flaccid with no detectable resistance to flexion/extension of the limb and impaired when the limb was able to move but not support the rat's normal posture. Tone was graded as normal when the rat had no visible limb weakness (normal symmetric posture and gait). Rats were typically reassessed at 2, 5, 10, 15, and 30 min after drug administration of the spinal agent.

**AUTONOMIC FUNCTION**

Under halothane anesthesia, neurologically normal rats with chronic intrathecal catheters were prepared with tail artery catheters (PE-50). BP and HR were continuously recorded with a Grass Model 79D Polygraph (Quincy, MA). Rats were allowed to recover for a minimum of 15 min, after which they received the intrathecal treatment.

**DRUGS AND INTRATECHAL INJECTIONS**

Morphine sulfate (Merck, West Point, PA) and lidocaine HCl (Astra, Westborough, MA) were dissolved in 0.9% saline. Bupivacaine HCl was commercially prepared, each milliliter containing 5 mg drug and 8 mg sodium chloride and with pH adjusted to 4.5–6.5. All drugs or drug combinations were administered in a single injection volume of 10 μl followed by a flush of 10 μl 0.9% saline, unless otherwise stated.

**EXPERIMENTAL PROTOCOLS**

**Motor Function**

In separate groups of rats, systematic examination of the motor function of the animals were made as described
above in the following groups: 1, 10, and 30 µg morphine; 25 and 75 µg bupivacaine; and 100 and 500 µg lidocaine, alone and with combinations of 100 and 500 µg lidocaine with 1 and 10 µg morphine and of 25 and 75 µg bupivacaine with 1 and 10 µg morphine. The combination doses were made with pretreatment of the rat with morphine and 10 min later with the local anesthetic. This timing was done to ensure that the time of peak pharmacologic action for spinal morphine approximately coincided with that of the more rapidly acting local anesthetics. Rats were then typically assessed at 2, 5, 10, 15, and 30 min after the second spinal injection. We thus sought to optimize the chance of detecting whether spinal morphine altered the severity of the motor blocking effects of the spinal anesthetics. In each case, the several drug treatments were carried out in separate groups of rats.

Antinoception

Several independent sets of experiments were carried out to assess the effects of local anesthetics alone and in the presence of morphine on the hot plate and paw pressure response.

EXPERIMENT 1: The effects of 100 and 500 µg intrathecal lidocaine and 25 and 75 µg bupivacaine alone on the hot plate and paw pressure latency were assessed as soon as there was recovery of normal motor function (see below).

EXPERIMENT 2: Based on the preceding studies outlined above characterizing the time course of the motor impairing effects of the anesthetics, the effect of 100 µg lidocaine and 25 µg bupivacaine were examined with a minimally effective dose of intrathecal morphine (1 µg). These doses of local anesthetic were chosen as they produced only a modest, transient change in motor function. To allow the time of peak morphine effect to coincide with the period when local anesthetic motor effects as defined by the motor index were not different from control, lidocaine was given 17 min after the spinal injection of either saline or a just minimally effective dose of morphine (1 µg), while bupivacaine was given concurrently with morphine (1 µg).

EXPERIMENT 3: To further explore the nature of the spinal anesthetic-morphine interaction, dose response curves for 0.03–10 µg intrathecal morphine were carried out either with the injection of morphine alone or with the concurrent injection of 25 µg bupivacaine.

EXPERIMENT 4: To explore the morphine-local anesthetic interaction, dose response curves were carried out with 2.5–25 µg bupivacaine alone or concurrently with 0.3 µg morphine. Concurrent dose response studies were not carried out with lidocaine.

Blood Pressure and Heart Rate

The effects of intrathecal bupivacaine on BP and HR in rats pretreated with intrathecal saline or morphine were studied. Serial injections of 25, 75, and 150 µg bupivacaine followed at 20, 30, and 40 min, respectively, after the pretreatment injection of either 1 or 30 µg morphine or saline. Bupivacaine was administered at a concentration of 5 µg/µl.

CLEARANCE OF SPINAL MORPHINE AND INULIN

Radiolabelled solutions of 0.1 µg/µl morphine and 0.1 µg/µl morphine with 2.5 µg/µl bupivacaine were prepared, each containing 3H-morphine (140,000 DPM) and 14C-inulin (95,000 DPM) per 10 µl. Thirty-five rats were randomized to receive 10 µl of either solution. Twenty-three rats were sacrificed at 30 min after drug injection, and 12 were sacrificed at 120 min. At the time of sacrifice, anesthesia was induced with halothane. Four milliliters of blood was extracted by direct cardiac puncture and aspiration through a 21-G needle, followed by decapitation. Blood was transferred to an EDTA (K3) vacutainer tube. At the appropriate interval, spinal cords were removed intact by hydraulic extrusion. This is accomplished following decapitation by inserting an 18-G blunt needle into the spinal canal in a cephalad direction after sharp dissection of the spinal canal at the levels of the L4–5 vertebrae. The needle is attached to a 5-ml syringe filled with 0.9% saline. The spinal cord is then readily extruded rostrally by the forceful injection of saline into the spinal canal. Spinal cords were blotted dry and immediately placed on a block of dry ice. When frozen, the cords were divided into segments of approximately equal lengths (cervical, thoracic, and lumbar) and stored at -20º C for 24–48 h.

To determine whether residual free drug was retained in the extraspinal, subdural space, after extrusion, the dura of four spinal cords was carefully removed. The cord was then rinsed in 0.9% saline to clear any superficial drug, blotted, and was placed on the dry ice.

To determine whether the extrusion procedure altered the drug distribution, the entire spine of four rats was rapidly dissected out from the level of the crest of the ilium (L4) to the point of decapitation (C1). The vertebral column was then divided into thirds by cutting at the point of the first and last ribs. The spinal cord segments were then removed intact, dura removed and rinsed in fresh 0.9% saline, and frozen by placing on dry ice.

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Spinal cord segments were prepared for radioactivity counting by defrosting, weighing, mincing, and solubilization (1 ml 0.5M Protosol tissue and gel solubilizer; Du Pont-Cat. No. NEF-935, Boston MA; incubation for 24 h at 55º C), and decolorization (100 µl of 30% H2O2).
The solution was allowed to cool to room temperature and 10 ml of toluene base scintillation solution (Econo-Melt-Dupont Cat. No. NEF-941, Boston, MA) was added.

Blood samples were centrifuged and plasma removed, and volumes were measured and transferred to liquid scintillation vials to which 10 ml of liquid scintillation solution (EcoLite-Cat. No. 882475-ICN Biomedicals, Costa Mesa, CA) was added. Counting was started after 2 h on a Packard Tri-Carb 1200CA liquid scintillation analyzer (Downers Grove, IL). Each sample was counted twice during 10 min in the spectrum of photoluminescence of $^3$H and $^{14}$C, using computerized automatic window tracking and gain restoration. Background spectrum correction was employed. Each sample was monitored for counting efficiency (detection of quenching), and the detected counts per minute were corrected to disintegrations per minute (DPM). These values were then used to determine morphine equivalents in plasma or spinal cord tissue. To calculate the normalized ratio of $^{1}$H-morphine/$^{14}$C-insulin for each plasma or tissue sample, the sample DPM1/DPM2 was corrected by multiplying by the corresponding DPM2/DPM1 ratio of the stock solution. Thus, the normalized ratio of morphine to insulin in the injectate was 1. Ratios less than 1 indicated that relatively more morphine was cleared than insulin.

**Data Analysis**

**Motor Function**

To analyze the effects of drugs on motor function, the individual motor scores (maximum possible score = 16 at each observation) for each rat made at the five measurement times were cumulated and divided by the maximum possible motor score (16 x 5 observations = 90) to give the percent of the maximum possible motor function score (percent maximum motor score). Values approaching 100% indicated normal function, and lesser values reflected increasing degrees of motor dysfunction. Statistical analysis were carried out on the percent maximum motor score.

**Noception**

For the noceptive tests, response latency data for each rat were converted to the percent of maximum possible effect (%MPE), where: MPE = (postdrug value - baseline value)/(cutoff value - baseline value) x 100%. The highest value for the %MPE and the area under the %MPE time course curve (AUC of the %MPE: derived by the trapezoidal integration method) was determined for each rat. Data analyses were performed on the MPE and AUC values.

**Statistical Analysis**

For statistical analysis of the data, randomized, one-way analysis of variance was employed. If statistical significance was observed, between-group comparisons were accomplished with a Newman-Keuls test. Individual comparisons were carried out where appropriate with paired or unpaired $t$ tests. Statistical significance was assumed if critical values reached a $P < .05$ level of probability. For log dose response curves (with %MPE and the AUC), linear regression analysis and values (with 95% confidence intervals) for the ED$_{50}$ and slope of the dose-response curves were derived using the graded dose-response: pharmacologic calculation system 4.0 computer program. The ED$_{50}$ value reflects that dose that yields an MPE of 50%. Analysis of the motor score data was accomplished by the use of the Kruskal-Wallis analysis of variance by ranks. Individual comparisons were carried out with the Mann-Whitney test for independent samples. Significance values for group size smaller than 20 were obtained from Siegel.

To assess the nature of the bupivacaine-opiate interaction on noception, the fixed dose analysis as described by Tallarida was employed. In this, the null hypothesis is that interaction between each of several doses of morphine and a fixed dose of the local anesthetic is respectively the sum of the effect produced by the two drugs alone (i.e., additivity). The theoretical dose response curve produced by morphine in the presence of local anesthetic is then determined and the ED$_{50}$ value for this theoretical curve calculated. The ED$_{50}$ value for the experimentally determined dose response curve carried out in the presence of the fixed dose of local anesthetic is then calculated along with its 95% confidence interval. If the confidence interval of the experimentally determined curve does not overlap with the ED$_{50}$ of the theoretical curve based on additivity, then the local anesthetic has by definition produced an effect that is greater than additive, or synergistic. The ratio of the ED$_{50}$ of the experimentally derived curve to the ED$_{50}$ of the theoretical curve of additivity indicates the minimum degree of potentiation. This approach is the method of choice in the event that one of the agents does not produce an ED$_{50}$ when given alone (i.e., in this case, the local anesthetic).

**Results**

**Motor Function**

According to the motor scoring system outlined above, all rats used in these studies showed a motor function score of 16, i.e., normal function prior to treatment. The results of examining motor function at 2, 5, 10, 15, and 30 min after spinal injection of morphine, lidocaine, and
bupivacaine, alone and with morphine, are presented in table 1 as the percent maximum motor score.

**Morphine**

Intrathecal morphine alone (0.1 μg–30 μg) was not found to affect motor function in any detectable fashion; motor function scores were uniformly 16 for the 30-min post-injection observation period at all doses, giving a percent maximum motor score of 100.

**Lidocaine**

Intrathecal lidocaine produced a very rapid (20–30 s in onset), transient motor block of the hindlimbs, which was dose-dependent over the range of 100–500 μg (table 1). Thus, at 100 μg, 6 of 12 rats had mild impairment of hindlimb function that disappeared by 2 min after injection; i.e., the motor score at 2 min for each rat was essentially normal = 14 ± 2. At this time, the animal could easily bear weight and showed only a modest weakness of the hind limb placing and stepping response. At 500 μg, 12 of 12 rats had 5–15 min of flaccid paralysis of the hindlimbs and motor function scores of 6 ± 3 at 10 min and 15 ± 5 at 15 min. Motor blocks were reliably localized, such that even at the highest dose of intrathecal lidocaine (500 μg/10 μl), when a flaccid paralysis of the hindlimbs was observed, forelimb function was unaffected. During the recovery phase of the block, animals would typically display an exaggerated tone of the hindpaws with ipsilateral clonic jerks of the hindlimb being evoked by pressure applied to the paw.

**Table 1. Motor Function (Percent Maximum Motor Score)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Intrathecal Dose (μg)</th>
<th>Drug Alone</th>
<th>+MOR 1 μg</th>
<th>+MOR 10 μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOR</td>
<td>1</td>
<td>100 ± 0*</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100 ± 0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>100 ± 0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LID</td>
<td>100</td>
<td>93 ± 4</td>
<td>92 ± 5</td>
<td>91 ± 6†</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>65 ± 6‡</td>
<td>66 ± 4‡</td>
<td>64 ± 10‡†</td>
</tr>
<tr>
<td>BUP</td>
<td>25</td>
<td>91 ± 4</td>
<td>89 ± 6</td>
<td>92 ± 3‡</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>58 ± 9‡</td>
<td>61 ± 2‡</td>
<td>54 ± 12‡†</td>
</tr>
</tbody>
</table>

*Values are mean and SEM of the percent maximum motor score of 6–12 rats each; 100 = normal motor function during the post-injection interval, with lower numbers indicating greater and longer lasting decrements in motor function.
† Comparison across anesthetic alone and anesthetic +1 μg and +10 μg MOR accomplished using a Kruskal-Wallis test, indicated no differences (P values ranged from .4 to .6 level of significance).
‡ High anesthetic dose motor function exceeds lower anesthetic dose percent maximum motor score; P < .05 (Mann-Whitney test for independent samples).

**Bupivacaine**

Intrathecal bupivacaine produced a rapid, dose-dependent reduction in hindlimb motor function over the range of doses of 25–75 μg (table 1). At 25 μg, all rats showed a rapid and transient hindpaw weakness, with a modest motor dysfunction observed at 2 min (motor score = 11 ± 3) and normal scores at 15 min (motor score = 14 ± 1). Thus, at this time, the rat displayed complete ability to bear weight and ambulate, but showed mild weakness of the placing and stepping response. After 75 μg, a significant block was observed with full recovery occurring between 15 and 30 min. The time course of this blockade after the high dose (75 μg) is presented in figure 1.

**Morphine and Local Anesthetic**

The addition of 1 or 10 μg intrathecal morphine to 25 or 75 μg intrathecal bupivacaine or to 100 or 500 μg lidocaine had no effect upon the degree or the duration of the change in motor function as indicated by the percent maximum motor score induced by the local anesthetic (table 1). Figure 1 presents the temporal changes in motor function scores for 75 μg bupivacaine alone or in the presence of 1 μg morphine and emphasizes this lack of effect on the time course of the change in motor function.

**Figure 1. Motor function score followed over time after spinal drug administration. Data are presented as mean ± SEM. Groups: MOR 1 + BUP 75, n = 10; BUP 75, n = 8 rats.**

**ANTINOCEPTION**

**Morphine**

Intrathecal morphine administration resulted in a dose-dependent increase in response latency in both the paw
pressure and hot plate tests, the \( ED_{50} \) with 95% confidence intervals being 1.1 \( \mu \)g (0.8–1.4) and 1.2 \( \mu \)g (0.7–1.9), respectively. Antinociceptive effects peaked around 15–30 min and were back to baseline within 120 min after a 1-\( \mu \)g dose (fig. 2).

**Local Anesthetics**

The intrathecal injection of 100 \( \mu \)g lidocaine (fig. 2) or 25 \( \mu \)g bupivacaine (fig. 3), tested at the earliest times after injection when the animals could maintain at least minimum support of the hindlimbs (2 min; see above), had no statistically significant effect upon hot plate latency or paw pressure escape threshold \( (P > .10) \). Similarly, we failed to see changes in hot plate and paw pressure responses with higher doses of intrathecal lidocaine (500 \( \mu \)g) or bupivacaine (75 \( \mu \)g) when testing was initiated at a time (15 min) after the anesthetic injection when minimal motor impairment was noted \( (i.e., \) at 15 min, motor scores of 13–16 for both agents).

**Morphine and Local Anesthetics**

The addition of 100 \( \mu \)g intrathecal lidocaine to rats pretreated with a low dose of intrathecal morphine (1 \( \mu \)g) resulted in a clear increase in the %MPE and AUC in both the hot plate and paw pressure tests (fig. 2 and table 2). The addition of 20 \( \mu \)l 0.9% saline had no effect on
the antinoceptive time course of intrathecal morphine (1 μg).

The concurrent spinal administration of 25 μg bupivacaine, a dose that had essentially no effect on either motor or antinoceptive measure, with morphine resulted in a significant augmentation of the effects of a low dose of intrathecal morphine on the hot plate and paw pressure (fig. 3).

In the systematic dose response analysis, 25 μg bupivacaine resulted in a significant leftward shift of the log dose response curves for both peak effect and for the area under the curve for morphine on both the hot plate (fig. 4) and the paw pressure (fig. 5) tests (table 3). Dose response curves carried out with intrathecal bupivacaine alone and with the concurrent administration of 0.3 μg intrathecal morphine are presented in figures 6 and 7. As indicated, there was a similar significant potentiation of the peak antinoceptive effect and the area under the curve at doses of either drug which yielded minimal effects when given alone.

These results, showing the significant effects of otherwise inactive doses of either bupivacaine or morphine, suggest a potent synergistic interaction. Calculation of the ED₅₀ of the morphine curves for theoretical additivity revealed that they fell outside the 95% confidence interval of the experimental curves derived for intrathecal morphine on the hot plate or paw pressure test (table 3). The respective dose ratios for the experimentally determined MPE-dose response curve as compared to the theoretical curve based on simple additivity of the effects produced by 25 μg bupivacaine alone, were 5.0- and 2.7-fold, respectively. For the % AUC versus dose, these ratios were 11.5 and 5.9, respectively. Were there no synergy, these ratios would be 1.

**Naloxone Antagonism**

Using the paradigm as described in figure 3, intrathecal injection of 1 μg morphine and 25 μg bupivacaine at time 0, followed by 0.3 mg/kg subcutaneous naloxone at time 17 min, resulted in a complete reversal of the elevated hot plate latency and paw pressure threshold otherwise

![HOT PLATE TEST](image)

**Fig. 4.** The maximum value of the percent MPE time course curve (top) and the AUC of the percent MPE time course curve (bottom) in the HP test as a function of the log of the intrathecal-MOR dose (alone or co-administered with intrathecal-BUP (25 μg). Data are presented as mean ± SEM; n = 3–9 rats/pool. Values for the slopes, ED₅₀, and potency ratios are presented in table 2.
TABLE 3. ED₉₀ Values and Slopes for the % MPE and % of the Maximum Possible Area under the Curve (AUC) with 95% Confidence Intervals for Intrathecal Morphine and Intrathecal Morphine + Bupivacaine (25 µg) on the Hot Plate and Paw Pressure Tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Intrathecal Drug Treatment</th>
<th>Potency Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MOR (+BUP 0.25 µg)</td>
<td></td>
</tr>
<tr>
<td>Hot plate test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%MPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED₉₀ (µg)</td>
<td>0.25 (0.18–0.42)*</td>
<td>1.72 (0.93–2.01)</td>
</tr>
<tr>
<td>Slope</td>
<td>57 (37–77)</td>
<td>44 (30–59)</td>
</tr>
<tr>
<td>%AUC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED₉₀ (µg)</td>
<td>0.93 (0.51–1.62)</td>
<td>10.8 (4.0–29.0)</td>
</tr>
<tr>
<td>Slope (/10)</td>
<td>893 (571–1215)</td>
<td>4970 (329–665)</td>
</tr>
<tr>
<td>Paw pressure test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>%MPE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED₉₀ (µg)</td>
<td>0.28 (0.21–0.44)</td>
<td>1.1 (0.8–1.4)</td>
</tr>
<tr>
<td>Slope</td>
<td>50 (39–60)</td>
<td>53 (44–63)</td>
</tr>
<tr>
<td>%AUC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ED₉₀ (µg)</td>
<td>1.10 (0.49–2.22)</td>
<td>6.9 (3.1–15)</td>
</tr>
<tr>
<td>Slope (/10)</td>
<td>850 (438–1222)</td>
<td>582 (400–764)</td>
</tr>
</tbody>
</table>

Values represent analysis of figures 4 and 5. Ranges are given in parentheses.
* ED₉₀ with 95% confidence interval.

observed after the concurrent administration of intrathecal morphine and bupivacaine (hot plate %MPE: 92 ± 4 vs. 12 ± 6 with naloxone, P < .05, unpaired t test; paw pressure %MPE: 72 ± 11 vs. 3 ± 5 with naloxone, P < .05, unpaired t test; n = 8). Naloxone alone was without effect upon hot plate or paw pressure responses.

**Blood Pressure and Heart Rate**

In chronically prepared, unanesthetized rats, the baseline heart rate and mean arterial blood pressure (mean ± SD) was 402 ± 35 beats/min and 108 ± 10 mmHg, respectively. The intrathecal administration of either saline, 1 µg morphine or 30 µg morphine had no effect on either measure. Subsequent intrathecal bupivacaine administrations had no significant effect on heart rate in any group at any time (range 363–423). Intrathecal bupivacaine caused a dose-dependent drop in mean arterial blood pressure that was statistically significant at the 150-mg dose in all three groups (repeated measures analysis of variance and Newman-Keuls; fig. 8).

**Spinal Cord Morphine Clearance**

Following 1 µg intrathecal morphine or 1 µg morphine/25 µg bupivacaine, the plasma morphine concentrations were similar at 30 min (0.66 ± 0.05 ng/ml vs. 0.70 ± 0.07 ng/ml; n = 12). At 120 min, morphine concentrations were also similar (0.44 ± 0.02 ng/ml vs. 0.43 ± 0.05 ng/ml; n = 6).

The μM-morphine/14C-inulin ratio in plasma was similar for both groups. The means ± SEM at 30 min were: 0.45 ± 0.04 for 1 µg morphine/25 µg bupivacaine and 0.39 ± 0.03 for 1 µg morphine alone; n = 12. At 120 min the ratios were: 0.26 ± 0.02 for 1 µg morphine/25 µg bupivacaine and 0.25 ± 0.01 for 1 µg morphine alone; n = 6.

With regard to spinal tissue, several observations were made. First, spinal morphine concentrations at 30 min after spinal injection were the same in those spinal cord segments harvested free of dura and rinsed in 0.9% saline whether the 1 µg morphine was delivered alone or with 25 µg bupivacaine. Second, segmental morphine concentrations were the same whether the cords were extruded whole or if division into segments took place while the segments were yet within the spine. Therefore, these data have been pooled (fig. 9). Third, those cords in which the dura was not removed had higher sample morphine concentrations in the group that was co-administered in-

![Fig. 5. The maximum value of the percent MPE time course curve (top) and the AUC of the percent MPE time course curve (bottom) in the PP test as a function of the log of the intrathecal-MOR dose (alone or co-administered with intrathecal-BUP [25 µg]). Data are presented as mean ± SEM; n = 3–9 rats/point. Values for the slopes, ED₉₀, and potency ratios are presented in table 2.](image-url)
duration of which are dose- and concentration-dependent. Specific examination of the analgesic action of these spinal local anesthetics using escape behavior evoked by thermal (hot plate) and mechanical (paw pressure) stimuli revealed that, in the periods immediately after recovery of motor function, there were no changes indicative of a selective alteration in the nociceptive threshold. This finding is in accord with that of others who examined the spinal action of lidocaine and bupivacaine in this model\(^{19}\) (see also reference 21).

In contrast, in the present studies, doses of intrathecal lidocaine or bupivacaine at doses and times at which no behavioral effect was detectable were able to significantly augment the antinociceptive activity of intrathecal morphine in the hot plate and paw pressure tests, as manifested by increases of both peak antinociceptive effect and dur-

FIG. 6. The maximum value of the percent MPE time course curve (top) and the AUC of the percent MPE time course curve (bottom) in the HP test as a function of the log of the intrathecal-BUP dose (alone or co-administered with intrathecal-MOR [0.3 μg]). The time course was limited to 60 min. MOR alone is also shown. Data are presented as mean ± SEM; \(n = 5-11\) rats/point. \(\ast P < .01, \ast\ast P < .05\) compared with MOR 0.3 μg alone and BUP alone (one-way ANOVA, Newman-Keuls, all groups).

Intrathecal bupivacaine (25 μg; \(P = .026\), unpaired, two-tailed Student's \(t\) test; fig. 9).

With regard to the tissue morphine/inulin ratio, the coadministration of 25 μg intrathecal bupivacaine resulted in lower mean cord \(^{3}H\)-morphine/\(^{14}C\)-inulin ratios in spinal cords where the dura was not removed. This was statistically significant at 120 min (\(P = .032\), unpaired, two-tailed Student's \(t\) test; fig. 10). Removal of the dura and rinsing the cords in saline had no effect upon the ratios. The \(^{3}H\)-morphine/\(^{14}C\)-inulin ratios were significantly lower in the cords harvested at 120 min than at 30 min.

**Discussion**

Intrathecal local anesthetics result in a reversible motor dysfunction and sensory anesthesia, the magnitude and

FIG. 7. The maximum value of the percent MPE time course curve (top) and the AUC of the percent MPE time course curve (bottom) in the PP test as a function of the log of the intrathecal-BUP dose (alone or co-administered with intrathecal-MOR [0.3 μg]). The time course was limited to 60 min. MOR alone is also shown. Data are presented as mean ± SEM; \(n = 5-11\). \(\ast P < .01, \ast\ast P < .05\) compared with MOR 0.3 μg alone and BUP alone (one-way ANOVA, Newman-Keuls, all groups).
ration of action. Thus, for example, the duration of the motor block produced by even high doses of spinal lidocaine (500 μg) or bupivacaine (75 μg) alone was less than 15 min, yet the lower doses of lidocaine (100 μg) and bupivacaine (25 μg), which produced only a minimum and at most a transiently detectable motor dysfunction, significantly increased the antinociceptive effects of a low dose of intrathecal morphine (1 μg) for periods of 1–2 h. Similarly, concurrent injection of doses of 8 μg bupivacaine and 0.3 μg morphine, which are 0.3 times a dose that had no motor effect and 0.1 times the ED₅₀, respectively, jointly yield a significant increase in the antinociceptive effect. This interaction is fully consistent with a synergic interaction between the spinal actions of these two classes of agents. This synergy was confirmed by the fact that the ED₅₀ for the experimentally determined dose response curve for morphine carried out in the presence of a dose of bupivacaine that was without effect, did not overlap the theoretical dose response curve of additivity for either the hot plate or paw pressure.

Importantly, the present work emphasizes that this augmentation is functionally selective for sensory processing. Thus, even when the morphine was given such that the time of peak pharmologic effect overlapped with the time of peak local anesthetic motor block, there was no enhancement in motor dysfunction even when a quantitative index of motor function was employed. Similarly, morphine failed to alter the sympathetic activity of the spinal local anesthetic.

**Mechanisms of Local Anesthetic and Opioid Interactions**

**Selective Anesthetic Effects on Small Afferents**

While differences in drug diffusibility and conduction safety factors associated with large versus small diameter afferents have been postulated to explain the characteristics of spinal anesthetic action, these hypotheses have generally been based on the characteristics of anesthetic actions on peripheral nerves and the relevance of...
these mechanisms for spinal effects remain speculative. For example, the augmentation observed in the present experiments was produced by both lidocaine and bupiva-
caine. Yet, on peripheral nerves, lidocaine has been shown to produce a preferential block of myelinated fib-
ers, whereas bupivacaine has a predilection for unmy-
elinated axons. In addition to these variables, several issues relevant to the spinal route of administration should be considered.

Altered Spinal Clearance

Spinal anesthesia with bupivacaine has been shown to decrease local spinal cord blood flow 27–34% in rats32 and 26–47% in dogs.33 Both investigators attribute the decrease in spinal cord blood flow to the unique effects of bupivacaine on the regional circulation of the spinal cord that is distinct from the accompanying decrease in mean arterial pressure. This hypothesis is tenable, in view of the fact that spinal lidocaine has been shown to have no effect34 and tetracaine has been shown to increase spinal cord blood flow by 62%.35 Therefore, one could post-
tulate that bupivacaine may decrease the rate at which morphine is cleared from the spinal cord. However, the augmentative interaction observed occurred with doses of bupivacaine much lower than those known to affect spinal cord blood flow. Moreover, while there were higher levels of morphine in the subdural space of animals receiving both bupivacaine and morphine (as measured in the intact cord), tissue morphine levels were unaltered by the coinjection of bupivacaine (as measured in those animals in which cords were cleared of their dura and immersed in saline). This finding is consistent with the lack of differences in the plasma levels of morphine in the several treatment groups. Therefore, we believe that the observed potentiative interaction is not likely due to changes in spinal morphine disposition.

Altered Activity at the Sodium Channel

It is conceivable that morphine may serve to facilitate the effects of the local anesthetics on the voltage sensitive sodium channel. The hypotension and motor weakness associated with the spinal action of local anesthetics is typ-
ically ascribed to the blockade of voltage sensitive sodium channels in efferent autonomic and motor nerves.36 Spinal opioids have no detectable effect upon resting blood pres-
sure or motor function, even at maximal antinociceptive doses.37 Polar opioids such as morphine applied directly to spinal nerves have not been shown to alter nerve con-
duction, though at high concentrations lipid soluble agents such as fentanyl can reduce action potential height.38,39

In the present studies, the administration of intrathecal morphine did not alter the blood pressure response to

intradural bupivacaine given over a wide range of doses. Similarly, at the 25-μg dose of bupivacaine, at the time of testing, there was no evidence of motor block, and motor function was not altered in the presence of mor-

phine at doses that induced a powerful antinociception. These several considerations suggest that, if the mor-

phine/local anesthetic interactions reflected a general augmentation of the local anesthetic interaction with the sodium channel, then this augmentation should be seen on all aspects of local anesthetic action, including motor and blood pressure. Failing these possibilities, we conclude that the interaction does not represent an exacerbation of the general effects of local anesthetics on the relevant voltage-sensitive ion channels.

Opioid Receptor Interaction

It is possible that local anesthetics may interact with the opioid receptor. While failure to alter the effects of bupivacaine alone with naloxone suggests the motor ef-
fcts are not mediated by such a mechanism, the synergy between morphine and bupivacaine could conceivably arise from such an interplay. Tejwani and colleagues32 have suggested this alternative. Yet, such an explanation appears unlikely, given that even micromolar concentra-
tions of bupivacaine fail to alter the binding of 3H mor-

phine in spinal cord. While those authors argue the pos-
sibility that the effects may reflect an interaction at a kappa receptor, this also appears unlikely in light of the lack of a significant effects of kappa receptors in the rat spinal cord on the hot plate test.40

physiologic mechanisms in the dorsal horn

After spinal delivery, the local anesthetic may interact with several distinct anatomic elements that can alter the transmission of afferent input.

blockade of long tract transmission

Though it is commonly accepted that spinal anesthetics act by an effect upon intradural nerve roots, several lines of evidence including distribution of labeled drug and the sensory consequences of spinal anesthesia has sup-
ported the possibility that local anesthetics may alter transmission in the superficial long projection tracts with myelinated axons that travel in the ventrolateral fu-
niculus.41–43

Effects in the Dorsal Root Entry Zone

The spinal afferents enter the dorsal horn in the dorsal root entry zone. The small afferents enter directly and

* Yaksh TL: Unpublished observations.
ramify widely to terminate in the substantia gelatinosa of the superficial dorsal horn as well as, to a lesser degree, in deeper laminae.\textsuperscript{44} Here, the afferent telodendria make synaptic contact with the dendrites of second order neurons. Binding studies have emphasized that opioid receptors are located presynaptically on these small afferent terminals and these receptors mediate the inhibition of release of C-fiber peptide neurotransmitters (such as substance P and calcitonin gene related peptide) by the blockade of the activation of voltage sensitive Ca channels.\textsuperscript{5} Two points should be emphasized. First, axonal branching routinely leads to conditions of impedance mismatching between the stem axon and its branches, which, along with the physics of electronic spread, suggests there is an increased probability of branch conduction failure (i.e., reduced safety factor; see also references 45–47). In addition, examination of the conduction through the branch point of the dorsal root ganglion reveals long-lasting subnormal periods,\textsuperscript{58} an event that would also contribute to a reduced safety factor for branch point conduction. Secondly, though we are aware of no data on sodium channel density at the central terminals of C-fibers in the spinal cord, it appears that in the distal terminals of at least large sensory axons, the density of voltage sensitive sodium channels, as defined by the sensitivity of generator potentials to tetrodotoxin, are diminished.\textsuperscript{49,50} These conditions, small diameter, branching, and lower Na channel density, are thus likely to be found in the central ramifying terminals of unmyelinated afferents in the superficial dorsal horn. We therefore hypothesize that low concentrations of local anesthetics in the dorsal root entry zone may serve to diminish transmission security because of the architecture of the C-fibers in this region. This decreased transmission security would lead to a reduction of the magnitude of afferent terminal depolarization, which would subsequently lead to a diminished activation of the voltage sensitive Ca channels. This reduction would in turn substantially facilitate the presynaptic inhibition by opioids of transmitter release from unmyelinated primary afferent terminals and thereby augment the apparent antinociceptive efficacy of spinal opioids. At present, we favor this hypothesis given the observation that these potentiative effects occur at local anesthetic concentrations that do not alter pain behavior, but that appear to diminish the safety factor of spinal information thus augmenting the actions of spinally active analgesics. Such mechanisms would argue that a synergic interaction also will be seen with other receptor agonists that appear to exert their spinal antinociceptive effects by a comparable mechanism, such as the \textalpha\textsubscript{2} receptor.\textsuperscript{51}

\textit{Dorsal Horn Actions}

In addition to the possible effects of local anesthetics on the conduction safety factor of the fine dendritic tree terminals of the ramified axons in the dorsal horn, local anesthetics also may interact with other receptors (e.g., nicotinic)\textsuperscript{52,53} and voltage gated channels, such as those for K and Ca.\textsuperscript{54,55} The direct effect upon Ca channels raises the speculation that local anesthetics might well serve to augment the presynaptic inhibitory effects of opioids, described above. It should be noted, however, that while dihydroxyridine channels have been reported to mediate transmitter release from dorsal root ganglion cells,\textsuperscript{56} lidocaine and bupivacaine have no effect upon 3H nitrendipine binding in brain,\textsuperscript{57} arguing against that specific interaction as a mechanism in these studies.

\textbf{Clinical Correlates of Concurrent Spinal Anesthetics and Opioids}

There is strong support in the clinical literature for the benefits of adding relatively low doses of narcotics to neuraxially administered local anesthetics. There is more controversy in the literature with regard to the merits of adding small, inactive amounts of local anesthetics to neuraxially administered opiates. In particular, to our knowledge there are no clinical reports that show that the addition of singularly inactive doses of local anesthetic to low doses of neuraxially administered opiates leads to a significant potentiation.

At present, systematic studies titrating different concentrations of opioid to local anesthetic against pain and side effects (autonomic blockade and motor function) remain to be reported, though the present animal studies suggest that a selective alteration in pain processing \textit{versus} autonomic or motor function may be achieved with the use of low doses of opioids and the appropriate anesthetic concentration.

An interesting aspect of the present studies is their potential significance for the interpretation of the clinically observed differential spinal block induced by local anesthetics, \textit{i.e.}, a selective effect on the reported perception of noxious (small axons) \textit{versus} non-noxious stimuli and motor function (large axons).\textsuperscript{51,54} In the present rodent studies, a differential block could not be detected with anesthetics alone on the several endpoints despite the use of several concentrations of either bupivacaine or lidocaine. Thus, at doses of anesthetic that did not alter motor or autonomic function, animals responded to the thermal and mechanical stimuli in a fashion suggesting that afferent input was sufficient to drive the normal behavioral response. However, at concentrations or times when the behavioral effects of the anesthetic alone could not be detected, the local anesthetic in the presence of lower

doses of spinal morphine produced a clear, dose-dependent change in spinal cord processing of high threshold information. Of particular significance, this augmented effect on sensory processing response was not due to a general exaggeration of axonal block, as the synergy occurred in the absence of any detectable effect upon motor or sympathetic function. Interestingly, it has been reported in humans that, during the receding phase of an epidural bupivacaine block, the injection of low doses of morphine can restore sensory levels. Such data, consistent with the present animal observations, argue that at concentrations of local anesthetics that may have no detectable effects alone on behavior result in changes in spinal sensory processing that are manifest in the presence of spinal opioid receptor occupancy (see below).

**Future Clinical Relevance**

Clinically, the neuraxial administration of low doses of bupivacaine that alone are subthreshold, together with spinally administered morphine, may have several potential advantages. First, lower concentrations of the opioid will lead to a reduction in the incidence of side effects that are mediated by redistribution (e.g., respiratory depression). Second, based on the hypothesis that coadministration of local anesthetics with neuraxially administered morphine leads to a greater analgesic effect for the given level of receptor occupancy, it may be postulated that the rate of opiate tolerance development may be decreased. Chronic pain patients receiving neuraxial morphine that develop tolerance may regain pain control by the addition of small amounts of local anesthetic. Third, the mechanisms of dyesthetic pain states evoked by low threshold afferents are not understood. One hypothesis currently suggests that this state represents a loss of inhibition on populations of dorsal horn wide dynamic range neurons which receive convergent A- and C-fiber input. Sensitivity of “injury pain” to spinal opioids reflects a joint action of the opioid receptors on the presynaptic C-fiber terminals and postsynaptically on the dorsal horn wide dynamic neurons. In contrast, the relative refractoriness of A-fiber-evoked dyesthetic pain states to opioids may reflect the specific association of opioid receptors on C- and not A-fiber terminals. It thus appears a reasonable hypothesis that low concentrations of local anesthetics may serve to alter large afferent input into the dorsal horn, and this in combination with the postsynaptic actions of the opioids, may serve as an effective therapeutic approach.

We caution that the favorable interaction may not occur with all local anesthetics and opiates. The local anesthetic 2-chloroprocaine appears to antagonize analgesia induced with epidural bupivacaine plus fentanyl by a mechanism that remains unclear. It has been reported that the addition of bupivacaine does not enhance the postoperative analgesia in knee surgery or abdominal surgery in patients receiving continuous epidural fentanyl. This may be due to fentanyl’s rapid systemic redistribution limiting its spinal effects. A second point that should be noted in combined drug administration is that drugs such as opioids and local anesthetics have different clearance times, and therefore continuous infusion will, at equilibrium, give anesthetic-opioid cerebrospinal fluid ratios that are different from those initially infused. In any case, the current animal and human literature clearly emphasizes that even the commonly used local anesthetics provide access to therapeutically useful mechanisms only just beginning to be understood.

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