Effect of Subarachnoid Bupivacaine Block on Anesthetic Requirements for Thiopental in Rats

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**Background:** Subarachnoid bupivacaine blockade has been reported to reduce thiopental and midazolam hypnotic requirements in patients. The purpose of this study was to examine if local anesthetically induced lumbar intrathecal blockade would reduce thiopental requirements for blockade of motor responses to noxious and nonnoxious stimuli in rats.

**Methods:** After intrathecal and external jugular catheter placement, rats were assigned randomly to two groups in a crossover design study, with each rat to receive either 10 µl of 0.75% bupivacaine or 10 µl of normal saline intrathecally. The doses of intravenously administered thiopental were calculated to ablate the eyelid reflex, to block the withdrawal reflex of a front limb digit, and to block the corneal reflex were compared. In two separate groups of animals, hemodynamic parameters and concentrations of thiopental in the brain were compared between intrathecally administered bupivacaine and saline.

**Results:** The thiopental dose required to block the described responses was decreased with intrathecally administered bupivacaine versus intrathecally administered saline from (mean ± SD) 40 ± 5 to 24 ± 4 mg/kg (P < 0.001) for the eyelid reflex, from 51 ± 6 to 29 ± 6 mg/kg (P = 0.005) for front limb withdrawal, and from 67 ± 8 to 46 ± 8 mg/kg (P < 0.01) for the corneal reflex. The concentration of thiopental in the brain at the time of corneal reflex blockade for the group given bupivacaine was significantly lower than in the group given saline (24.1 ± 2.6 vs. 35.8 ± 6.2 µg/g, P = 0.02).

**Conclusion:** This study demonstrates that local anesthetically administered lumbar anesthetic blockade decreases anesthetic requirements for thiopental for a spectrum of end points tested. This effect is due neither to altered pharmacokinetics nor to a direct action of the local anesthetic on the brain; rather, it is most likely due to decreased afferent input. (Key words: Anesthesia mechanisms; anesthetic interactions; balanced anesthesia; intrathecal; spinal anesthesia.)

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Placement of Catheters

The rats were anesthetized with 50 mg/kg ketamine and 12 mg/kg xylazine by intraperitoneal injection. Intrathecal catheters were implanted using the model described by Yaksh and Rudy. A 32-gauge catheter fused to PE-50 tubing was cleaned by immersion in 70% ethanol and flushed with sterile saline before use. The head and posterior neck were shaved and cleaned with iodine solution before surgery. The catheter was advanced from the atlantooccipital membrane 9 cm into the subarachnoid space and secured with the use of dental acrylic to the occiput. The trapezius muscle was sutured, and the catheter was again secured to the trapezius muscle. The volume of the intrathecal catheters ranged from 11 - 14 μl. The skin incision was closed with 9-mm wound clips, and the animals were allowed to recover 5 - 7 days. If any signs of motor weakness or sensory deficit were evident, the animal was killed with an overdose of halothane and not used in any aspect of the study.

After confirming that the rat had no neurologic deficit after intrathecal catheter placement (24 - 48 h later), a 9-cm-long PE-50 catheter for drug infusion was inserted 2 cm into the right external jugular vein, using the technique described by Herd and Barger. The free end of the catheter was exteriorized through the skin at the back of the neck, flushed with heparinized saline, and sealed until needed.

Behavioral Testing

Testing was conducted in a clear plastic box (20 × 24 × 45 cm) with no top to allow easy access to the rats. Rats were allowed to roam freely for 20 min in the box before testing, and testing was done individually. We selected one hypnotic and two nociceptive end points. The hypnotic endpoint was the ablation of the eyelid reflex to tactile stimuli. The right or left eyelid was stroked just above the eye with a cotton-tipped wooden applicator, and the response of the closing of the eyelid was graded as no response, decreased response, or normal response. The dose of thiopental required to block this response was recorded. The first nociceptive end point was blockade of the withdrawal reflex of a front limb to noxious stimuli. A painful clamp (160 g/4.5 mm²) was applied to the midphalanx of the first or second digit of the front left or front right limb for a maximum duration of 5 s. The dose of thiopental required to block limb withdrawal was recorded. Withdrawal within the 5-s period of clamp application was considered positive. The second nociceptive end point was blockade of the corneal reflex to noxious stimuli. The dose of thiopental required to eliminate the blink response to the application of a 4.56-mN von Frey filament to the cornea was recorded.

Although we considered blinding the investigator who was performing the testing, it was readily apparent which rats had received intrathecally administered bupivacaine and which had received intrathecally administered saline. Both corneal reflex testing and the upper limb digit pinch often caused the rat to move away using all its legs (if possible). Therefore, although preferable, we could not conceive of a reasonable manner of testing which would have allowed blinding.

Study Design

The study included four series of experiments with the following specific goals: (1) to study intrathecal bupivacaine–intravenous thiopental behavioral interactions; (2) to determine the effects of the applied dose of intrathecal bupivacaine on the basic hemodynamic parameters; (3) to determine the effects of the applied dose of intrathecal bupivacaine on concentration of thiopental in the brain; and (4) to assess the possibility of the rostral spread of bupivacaine after its intrathecal injection at the lumbar level.

Intrathecal Bupivacaine–Intravenous Thiopental Interaction Series. This series of experiments (n = 12) had a randomized crossover design. Each rat received the intrathecal injection of bupivacaine or saline followed by the intravenous infusion of thiopental. After a recovery period of ≥ 3 days, the other intrathecal agent, saline or bupivacaine (followed by the thiopental infusion), was administered. The measurements of behavioral end points were recorded before intrathecal injection and every minute during the infusion of thiopental until all responses were blocked.

All intrathecal injections were administered with a Hamilton (Reno, NV) 25-μl syringe and injected for ≈ 30 s. The intrathecal injection consisted of either 10 μl of 0.75% bupivacaine or 10 μl of saline plus 15 μl of saline (flush) separated by a small air bubble (a total volume of 25 μl). The effect of spinal anesthesia was confirmed with a lack of response to a painful pinch on the first digit of the hind limbs and a positive response at a digit of the fore limbs 90 s after the injection was completed. If the intrathecal injection of bupivacaine did not lead to bilateral lower extremity paralysis within 90 s, the animal was excluded from the study. Unilateral block occurred in three animals, and these animals were excluded from the study. Intravenous infusion of thiopen-
tal (25 mg/ml) was started 2 min after the intrathecal injection at 4 mg·kg⁻¹·min⁻¹. Each of the end points was assessed every minute until the response was blocked.

The doses of thiopental were chosen based on preliminary trials confirming that the thiopental doses for the blockade of the chosen end points could be differentiated and that blockade of corneal reflex would occur in <20 min (maximum duration of blockade after intrathecally administered 10 μl 0.75% bupivacaine in rats). Also, based on our preliminary work with intrathecal bupivacaine blockade in rats showing that all blocks were evident in ≦90 s, we chose the 2-min point to start the infusion of thiopental.

After each animal had been tested with both the intrathecally administered bupivacaine and the intrathecally administered saline, the animals were killed with an overdose of halothane. The position of the tip of the intrathecal catheter was detected in 11 rats. In six rats, 10 μl of methylene blue was injected and flushed with 15 μl of saline through the intrathecal catheter, and the distance of dye spread was measured.

**Hemodynamic Series.** Five rats were used in this series. They received either saline or bupivacaine, given intrathecally, in a randomized crossover manner. The systolic blood pressure and heart rate were recorded using the noninvasive tail cuff method. Animals were allowed to acclimate to the testing chamber for 10 min with the tail cuff in place before intrathecal injection. Measurements were made before and every 3 min after intrathecal injection for 20 min.

**Thiopental Concentration Series.** Twelve rats (n = 6 per group) were randomly given either 10 μl 0.75% bupivacaine or 10 μl saline, both intrathecally, and thiopental was infused at 4 mg·kg⁻¹·min⁻¹ until blockade of the corneal reflex was determined. These animals were decapitated, and the brains were excised (<2 min after decapitation). The brain tissue was transferred to plain glass tubes. The samples were mixed with a volume of distilled water (approximately five times the weight of the tissue) and homogenized using a Polytron Homogenizer (Brinkman Instruments, Westbury, NY). The homogenate was stored at 4°C until sent for analysis.

**Bupivacaine Concentration Series.** Five animals received an intrathecal injection of 10 μl 0.75% bupivacaine, followed by 15 μl saline. After bilateral lower extremity blockade was confirmed by the lack of response to painful stimuli applied to lower limb digits, intravenous infusion of thiopental was started at 4 mg·kg⁻¹·min⁻¹ and continued until the corneal reflex was blocked (≈ 10 min). These rats were then decapitated, and the brains were excised (<2 min after decapitation). The blood vessels and choroid plexus were removed to the extent feasible. The bony vertebrae were quickly dissected free from the body and sectioned into three equal parts (approximately at T1 and T10). Each section of spinal cord was dissected free from the surrounding bony structures. These sections of brain and spinal cord were homogenized as described previously and used to assess tissue concentrations of bupivacaine.

**Drug Assay**

Analysis of bupivacaine-containing tissue homogenate was performed by Quest Diagnostics (Cambridge, MA) using high-performance liquid chromatography with fluorescence detection. The coefficient of variation of this assay in their laboratory is approximately ±1% with an accuracy of 15% at the expected concentrations of bupivacaine and their minimum detection level at 0.1 μg/ml. The analysis of the brain specimens for concentrations of thiopental was performed by MED/TOX Laboratories, Inc. (St. Paul, MN) by a gas chromatography, flame ionization detection method. Their minimum detection level was 1.0 μg/ml, with accuracy reported to be ≈ 10%.

**Statistical Analysis**

A Student’s t test was used to compare means. A single-tailed, paired t test was used for comparison of thiopental dose requirements. Unpaired t tests were used to compare the concentrations of bupivacaine in the spinal cord and to compare the concentrations of thiopental in the brain. The Wilcoxon’s matched-pairs signed rank test was used to compare the hemodynamic data between the intrathecal saline and intrathecal bupivacaine groups. A probability value of <0.05 was considered statistically significant.

**Results**

The tips of all catheters dissected were found to lie at the lumbar vertebral level and in the subarachnoid space. One catheter was at the L1–L2 level, whereas the remaining catheters were approximately at the L3–L6 level. The methylene blue staining of the spinal cord was always <2 cm from the tip of the catheter.

Data are presented as the mean ± SD. The dose of thiopental required to block the described responses
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![Graph showing the effect of intrathecal bupivacaine on thiopental anesthetic requirements.](image)

**Fig. 1.** Effect of intrathecal bupivacaine block on thiopental anesthetic requirements. The intravenous dose of thiopental (given at 4 mg·kg⁻¹·min⁻¹) required to block the described responses is displayed on the vertical axis. Saline (10 µL) or bupivacaine (10 µL, 0.75%) was administered intrathecally in a crossover design. The dose of thiopental required to block the described responses was decreased with intrathecally administered bupivacaine by 39% for the eyelid reflex, 43% for front limb withdrawal, and 31% for the corneal reflex. Corneal reflex = blockage of the blink to corneal stimulation by a 4.56-mN von Frey filament; eyelid reflex = ablation of the eyelid closing to tactile stimuli; front limb withdrawal = blockage of the movement to clamping of a digit; *P < 0.001; **P < 0.005; ***P < 0.01.

was decreased with intrathecally administered bupivacaine versus intrathecally administered saline from 40 ± 5 to 24 ± 4 mg/kg (P < 0.001) for the eyelid reflex, from 51 ± 6 to 29 ± 6 mg/kg (P < 0.005) for front limb withdrawal, and from 67 ± 8 to 46 ± 8 mg/kg (P < 0.01) for the corneal reflex as displayed in figure 1.

The systolic blood pressure and heart rate measurements taken before and after bupivacaine and saline administration and revealed no differences between the two groups as displayed in figure 2.

The concentrations of thiopental in the brain for the bupivacaine and saline groups are displayed in figure 3. The concentration of thiopental in brain tissue was significantly lower (24.1 vs. 35.8 µg/g; P = 0.02) in the bupivacaine group compared with the saline group.

The concentrations of bupivacaine in the brain and spinal cord tissue after intrathecal injection of bupivacaine are displayed in figure 4. No bupivacaine was detectable in the brain nor in the cervical spinal cord. The laboratory reported the concentration of the homogenate, and these values were converted back to tissue concentration based on the weight of the sample and the amount of distilled water used to homogenize the sample. The lowest detectable concentration of bupivacaine by the laboratory assay was 0.1 µg/ml, which would yield a maximum concentration of 0.4 µg/g of tissue in the brain samples and 2.2 µg/g of tissue in the cervical cord samples; that is, it would have been possible to have this small amount of bupivacaine (or less) in the brain and cervical spinal cord and the assay would not have been able to detect it. There were no statistically significant differences between concentrations of bupivacaine in the thoracic versus the lumbar spinal cord tissue.

**Discussion**

Gustafsson et al.¹⁷ recently have characterized the anesthetic actions of thiopental in rats using responses to a variety of nonnoxious and noxious stimuli. They demonstrated that the difference among the doses of thiopental required to achieve the initial effect, the blockade of the righting reflex, and the ultimate anesthetic effect (blockade of abdominal muscle contraction to intubation) was fourfold. Of the wide spectrum of effects characterizing thiopental-induced anesthesia, we selected three end points for our study: One that reflects the hypnotic effect — ablation of the eyelid reflex to tactile stimuli — and two antinoceptive end points — blockade of the paw withdrawal reflex to digit clamping and corneal reflex ablation. In rodents, the loss of the righting reflex is a common index for assessment of the hypnotic effect of general anesthetic agents, but we could not use this end point because of hind limb paralysis after spinal administration of bupivacaine. In our experiments, the dose of thiopental for blockade of the eyelid reflex was 40 ± 5 mg/kg, and blockade of the limb withdrawal to noxious stimulation required an increase of ≈ 25% in the thiopental dose to 51 ± 6 mg/kg. This increase in dose is similar to the difference between intravenous (bolus) doses of thiopental necessary for blockade of the righting reflex and blockade of somatic motor response to tail compression in rats determined in an earlier study.¹⁸

Our study demonstrated that lumbar intrathecally administered local anesthetic blockade decreases (by 39%) thiopental hypnotic requirements in rats. This is consistent with results reported in several clinical studies, which have showed decreased hypnotic requirements (blockade of the response to a voice command) after spinal anesthesia¹² or epidural anesthesia.³

Our study also has examined the influence of intrathecally administered local anesthetic blockade on thiopental requirements for suppression of motor responses to...
noxious stimuli: Lumbar spinal bupivacaine decreased the thiopental requirements to block the corneal reflex and the front limb withdrawal response by 31% and 43%, respectively. It is noteworthy that spinal block reduced thiopental requirements to approximately the same degree for all three end points. A similar degree of modulation for the hypnotic and antinociceptive components of general anesthesia could indicate several possibilities. For example, hemodynamic changes caused by intrathecal blockade could lead to a change in general anesthetic requirements by various mechanisms, including the modification of thiopental distribution. Such an effect could have produced similar changes in the drug requirements for all components.

Fig. 3. Concentration of thiopental in the brain. Intrathecally administered bupivacaine (0.75%, 10 μl) versus intrathecally administered saline (10 μl). At the time of corneal reflex ablation, the concentration of thiopental in the brain was 33% lower in the bupivacaine group. *P < 0.02.
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![Graph showing concentration of bupivacaine in brain and spinal cord sections after intrathecal injection.](image)

Fig. 4. Concentrations of bupivacaine in the brain and cervical, thoracic, and lumbar spinal cord after intrathecal injection of bupivacaine (10 µl, 0.75%) at the lumbar spinal level. **0.4 µg/g** is the assay’s lowest detectable amount of bupivacaine for the brain samples; **2.2 µg/g** is the lowest detectable amount for the cervical spinal samples. No bupivacaine was detectable in the brain and cervical spinal cord samples.

of anesthesia. The results obtained in the hemodynamic series of the experiments, however, did not demonstrate any significant differences between the intrathecally administered bupivacaine and saline groups to support this possibility. It must be stated that the rats in the hemodynamic portion of the study were not given thiopental in addition to their intrathecal injections. It is possible that the combination of intrathecally administered bupivacaine and intravenously administered thiopental may have led to a decrease in blood pressure or heart rate, and our study did not evaluate this possibility.

The series that examined the concentration of thiopental in the brain indicated that the observed changes in thiopental requirements are most likely pharmacodynamic in nature. If pharmacokinetic factors were significant (e.g., spinal bupivacaine decreasing the volume of distribution of intravenously administered thiopental), then the differing doses of thiopental in the two groups of rats may have led to comparable concentrations of thiopental in the brain. Concentrations of thiopental in the brain were significantly lower in the bupivacaine group than in the saline group, suggesting that less thiopental was needed at the effector site. Thus, pharmacokinetic mechanisms that could have been responsible for changes in the thiopental requirements for different components of anesthesia are not likely.

One of the concerns with the previous clinical studies showing decreased anesthetic requirements after spinal anesthesia was the inability to confirm that local anesthetic agents did not migrate to the brain via the cerebrospinal fluid. The absence of detectable concentrations of bupivacaine in the brain or cervical spinal cord sections make the possibility of a direct effect due to the rostral spread of bupivacaine unlikely. In addition, although the spread of methylene blue in relation to the spread of isobaric bupivacaine in rats is unknown, the extension of dye did not exceed 2 cm from the tip of the intrathecal catheter. This is consistent with a similar finding of Yaksh and Rudy, who found a spread of only 2.5 cm from the tip of the catheter after a 10-µl injection. This is indirect evidence that there was not excessive cephalad spread of bupivacaine in the subarachnoid space.

Recent evidence has suggested that blockade of nociceptive movement by inhalational agents is largely determined at the spinal cord level. Anesthetic agents of all classes, including barbiturates, are known to depress excitatory transmission in the spinal cord, but it remains unclear how local anesthetic blockade at the spinal cord can modulate the action of general anesthetic agents at higher levels of the spinal cord and at the level of the brain. We suggest that the decrease in afferent input caused by the spinal block in turn leads to a decrease in descending facilitatory modulation, which acts at both the effecter sites of the hypnotic and the antinociceptive actions of thiopental. This would explain the parallel reductions in thiopental requirements for the different experimental end points. Several descending pathways have been described that may exert facilitatory influences on nociceptive flexion reflexes. It was also described that, in rats, pentobarbital-induced inhibition of the tail flick reflex can be enhanced by surgical transection of the spinal cord, indicating a role of descending excitatory modulation in the effect of barbiturates on a spinal nociceptive reflex. Although there has been much work reported regarding the role of descending spinal transmission in nociception, we are unaware of research looking at the change of descending activity related to local anesthetic-induced spinal blockade. Overall, a decrease of afferent input caused by the spinal block probably decreases a descending excitatory modulation, influencing both the hypnotic and antinociceptive effects of thiopental.

A possible criticism of our study is that we did not inject bupivacaine intravenously or intramuscularly in the saline group. We thought that systemic absorption after 10 µl bupivacaine administered intrathecally would have been insignificant. Therefore, we assume that the results seen here are not due to systemic effects...
of local anesthetic agents. Also, we didn’t measure body temperature during our experiments, and although slight core cooling may have taken place during the twenty minutes of spinal bupivacaine, it is unlikely. It is certainly unlikely that any temperature decrease occurred in 5 minutes, and the 40% reduction in thiopental dose for the eyelid reflex could not be due to hypothermia.

We have found a decrease in thiopental dose requirements for both hypnotic and antinociceptive effects after intrathecal bupivacaine blockade. This is not due to a direct effect of local anesthetic agents at the brain nor to changes in thiopental pharmacokinetics induced by spinal anesthesia. We suggest that the changes in anesthetic requirements may be due to the decrease in afferent input resulting in a reduction of descending excitatory modulation. Further work needs to be done to isolate the specific pathways, receptors, and mediators responsible for the changes seen.

References