Anesthesiology
1997; 86:1094–103

A Physiologic Assessment of Intrathecal Amitriptyline in Sheep

Sergio E. Cerda, M.D., Chuanyao Tong, M.D.,† Dwight D. Deal, B.S.,‡ James C. Eisenach, M.D.§*

Background: **Intrathecal injection of amitriptyline enhances antinociception from intravenous morphine and reduces neuropathic pain behavior in animals. This study represents part of a preclinical assessment of intrathecal amitriptyline to determine its safety for use in humans.**

Methods: **Low thoracic intrathecal, femoral, and pulmonary arterial catheters were inserted in 18 adult ewes, followed 96 h later by intrathecal injection of saline or 5 mg amitriptyline and by determination of spinal cord blood flow, hemodynamic variables, behavioral changes, cerebrospinal fluid concentrations of catecholamines and amitriptyline, and spinal tissue concentrations of amitriptyline. In six other ewes, low thoracic intrathecal and femoral arterial catheters were inserted and blood pressure and heart rate were measured after intrathecal injection of saline or 0.25, 1, or 5 mg amitriptyline. Four other ewes received cervical intrathecal injection of 5 and 10 mg amitriptyline, and antinociception was determined.**

Results: **Thoracic intrathecal injection of amitriptyline produced dose-dependent sedation but did not significantly affect spinal cord blood flow or hemodynamic variables. Spinal cord tissue concentrations of amitriptyline were 100 times greater in tissue near the tip of the thoracic intrathecal catheter compared with cervical cord tissue. Cerebrospinal fluid concentrations of catecholamines did not significantly change after amitriptyline was administered. Cervical intrathecal injection of 5 mg amitriptyline produced mild antinociception, whereas 10 mg produced intense sedation and, in one sheep, seizures and death.**

Conclusions: **Although other preclinical toxicity studies are necessary before introducing intrathecal amitriptyline for use in humans, this study did not reveal dangerous changes in blood pressure or spinal cord blood flow from this agent. (Key words: Analgesia, spinal: amitriptyline. Antidepressants: amitriptyline. Cerebrospinal fluid components: norepinephrine. Microspheres, colored. Receptors, spinal cord: N-methyl-d-aspartate. Spinal cord: blood supply; drug effects.)**

EXPERIMENTS in animals suggest that intrathecal injection of the tricyclic antidepressant amitriptyline may be useful for treating acute and chronic pain.[1,2] Systemically administered opioids produce analgesia in part by activating bulbospinal inhibitory pathways, which release serotonin and norepinephrine.[3] Spinal injection of reuptake inhibitors of these monoamines, such as amitriptyline, enhances analgesia from systemic opioid injection.[1] In addition, several animal models of neuropathic pain are sensitive to intrathecal injection of *n*-methyl-*d*-aspartate receptor antagonists.[4–6] Recently, amitriptyline has been shown to bind to *n*-methyl-*d*-aspartate receptors and antagonize effects of this receptor activation.[7–9] Intrathecal injection of amitriptyline in rats abolishes, in a manner similar to *n*-methyl-*d*-aspartate antagonists, inflammatory hyperalgesia.[2] These experiments in animals suggest that intrathecal injection of amitriptyline could reduce the dose of intravenous opioids needed to treat acute pain and could relieve chronic neuropathic pain.

Before intrathecal amitriptyline can be used in a clinical setting to treat acute or chronic pain, we must determine its safety. Included in this evaluation are examinations of animal behavior, histologic analysis of the spinal cord and blood flow, and determination of the effects of high concentrations of drug in the cerebrospinal fluid (CSF) surrounding the brain stem.[10,11]

This study assessed the effects of thoracic intrathecal administration of amitriptyline on spinal cord perfusion and hemodynamic variables and behavior, and of cervical intrathecal administration of amitriptyline on antinociception in conscious sheep. In addition we examined drug disposition by measuring concentrations of amitriptyline in CSF and spinal cord tissue near and distant from the site of injection and measured CSF catecholamine concentrations before and after spinal injection of this inhibitor of monoamine reuptake.

* Assistant Professor of Anesthesia, University of Chile, Santiago, Chile.

† Research Assistant Professor of Anesthesia.

‡ Research Assistant in Anesthesia.

§ Professor and Chair for Anesthesia Research.

Received from the Department of Anesthesia, The Bowman Gray School of Medicine, Winston-Salem, North Carolina. Submitted for publication September 18, 1996. Accepted for publication December 18, 1996. Supported in part by National Institutes of Health grant 48085.

Address reprint requests to Dr. Eisenach: Bowman Gray School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157-1009. Address electronic mail to: eisenach@bgsm.edu

Other ongoing studies not described here include histologic analysis of the spinal cord and examination of animal behavior after long-term exposure in rats and dogs.

Methods

After we obtained approval of the Animal Care and Use Committee, we studied 28 nonpregnant ewes of mixed Western breeds that weighed 40–50 kg in three different experiments.

Experiment 1: Spinal Cord Blood Flow and Hemodynamics after Intrathecal Amitriptyline (18 Sheep)

Surgery. Animals were fasted for 48 h and anesthesia was induced with 15 mg/kg ketamine given intramuscularly. The trachea was intubated and anesthesia was maintained with 1–2% halothane in 100% oxygen *via* mechanical ventilation. A low lumbar hemilaminectomy was performed, the dura mater exposed, and a single distal port 18-gauge catheter (Portex, Keene, NH) was inserted into the intrathecal space through a small hole and advanced 8 cm cephalad. The incision was closed and the catheter was secured firmly using sutures. All animals were standing up and eating and drinking normally within 4 h after surgery. Flunixin (1.1 mg/kg) was available after operation for behavioral signs of pain, such as not eating, rapid respiratory rate, attention to the incision site, or obvious distress; and intramuscular penicillin (2,000,000 U) was given daily after surgery.

On the third postoperative day, animals were anesthetized with halothane and polyvinyl catheters were inserted under direct vision into both femoral arteries, secured by sutures, and placed into a canvas pouch on the flank of the animal. A 7-French soft catheter was advanced under pressure waveform guidance into the left ventricle through the right internal carotid artery. A 7.5-French pulmonary artery catheter (Baxter, Irving, TX) was inserted using the Seldinger technique through the right common jugular vein under pressure waveform guidance into the distal pulmonary artery until capillary wedge recordings were verified. Catheters were secured and the animal was allowed to awaken from anesthesia and transferred to a metabolic cart. Intramuscular gentamicin (80 mg) and intravenous ampicillin (2 g) were administered after the catheter was inserted. Experiments were done the next day. The status of the animal was verified and noted in writing each morning by the investigators and veterinary faculty.

Experimental Protocol. Experiments were conducted with the sheep standing in a cage with a loosely fitting sling placed under the animal to prevent it from sitting during the study. Femoral and pulmonary arterial catheters were connected to pressure transducers (Viggo-Spectramed, Oxnard, CA) for continuous monitoring of systemic, pulmonary, and central venous pressures using a Grass (Quincy, MA) polygraph and online computer data acquisition system (MP100; Biopac Systems, Santa Barbara, CA). Average values were recorded at 1-min intervals. The left ventricular catheter was connected to a pressure transducer to verify location within the left ventricle or left atria before we began the experiment.

After 45 min of baseline recordings, colored microspheres were injected through the left ventricular catheter. Animals were randomized to receive saline or 5 mg amitriptyline by slow intrathecal injection, followed in 15, 30, 60, and 120 min by left ventricular injection of microspheres of a different color. This dose of amitriptyline was chosen in this toxicologic assessment to represent approximately 25–50 times the anticipated human dose (see Discussion). Each left ventricular injection consisted of 20 x 10^6presonicated colored microspheres (15.5 μm diameter; Ultrasphere, Los Angeles, CA). Reference sampling from both femoral arteries (8 ml/min) was begun 30 s before microsphere injection and lasted 120 s. At the time of each microsphere injection, cardiac output was determined by thermodilution in triplicate with injection of 5 ml iced dextrose solution, and arterial blood samples were obtained for blood gas tension and *p*H analysis using a Radiometer microanalyzer (Copenhagen, Denmark). Throughout the experiments, animals were observed for behavioral changes (refusal to eat, signs of agitation, sedation, vocalization, scratching, biting) or evidence of motor blockade (inability to stand unsupported or to stand with pressure applied downward on the hips). At the end of the experiment, CSF samples were withdrawn from the catheter for catecholamine and amitriptyline analysis by high-pressure liquid chromatography. Deep anesthesia was induced with pentobarbital (10 mg/kg given intravenously) followed by intravenous injection of saturated potassium chloride to produce cardiac standstill. A dorsal laminectomy from the lumbar to cervical spine was performed, the intrathecal catheter tip position was verified, and the entire spinal cord was removed to determine spinal cord blood flow and tissue

concentrations of amitriptyline. The spinal cord was divided in lumbar, thoracic, and cervical segments and the dura and pia mater was removed. Each segment was divided in the midline in two lateral parts and then the gray and the white matter were separated. Tissue samples were weighed and at least 2 g white and 1 g gray matter were separated to determine spinal cord blood flow. Tissue samples obtained near the tip of the intrathecal catheter and from the cervical cord were also dissected into gray and white matter, weighed, and stored at −70°C for amitriptyline assay. In addition a sample of each renal cortex was removed to document adequate mixing of microspheres in blood.

Determination of Spinal Cord Blood Flow. Tissue and reference blood samples were processed in our laboratory by one of the authors (D.D.D.) according to an established protocol.[12] Briefly, each tissue sample was digested by alkaline hydrolysis in a heated solution until all of the tissue was homogenized into suspension. The homogenate was centrifuged and the supernatant aspirated, leaving a small pellet containing the microspheres. This pellet was resuspended in the counting reagent (Microsphere; EZ-Trac System, Los Angeles, CA), sonicated for even microsphere suspension, and the microspheres in solution were counted using the Tracker 1000 EZ-Trac System. The Tracker 1000 is an integrated microscopic workstation using a 80-486 computer subsystem, true-color digital image acquisition, and custom-designed software that allows on-line computation of regional blood flow.

Spinal cord and renal blood flow, represented as milliliters per minute per 100 g tissue were determined by

$$Qt = (100 \times Qr \times Ct)/Cr$$

where Qt = tissue blood flow (ml/min), Qr = reference sample blood flow (ml/min), Ct = number of microspheres in the tissue sample, and Cr = number of microspheres in the reference sample.

Neurochemical Assays. Norepinephrine, epinephrine, and dopamine were determined by high-pressure liquid chromatography with electrochemical detection, as previously described.[13] The interassay coefficient of variation of this method for these catecholamines is less than 9%, and the lower limit of detection is 12 fmol for norepinephrine, 3.3 fmol for epinephrine, and 1.8 fmol for dopamine. Serotonin is not measured in this system.

For the amitriptyline assay of CSF samples, we used a high-pressure liquid chromatography method with ultraviolet detection.[14] Briefly, 100 μl internal standard (10 μg/ml imipramine) and 2 M NaOH were added to 1 ml CSF. After 15 min of shaking with hexane isoamyl alcohol and 15 min centrifugation at 3,000*g*, the organic phase was transferred and 0.2 M phosphate buffer was added. The mixture was vortexed and centrifuged for 15 min, the material from the organic phase was discarded, and 100 μl aqueous solution was injected into the high-powered liquid chromatography unit. Sensitivity of the method is 3 ng/ml in the original sample.

To determine amitriptyline concentrations in spinal cord, we modified the method described by Coudore *et al.*[15] Briefly, the tissue was homogenized in 0.1 M acetic acid, and the internal standard (200 ng imipramine) and HCO_3 (*p*H 10.5) were added to 1 ml of the homogenate. Extraction was done by shaking the mixture mechanically for 30 min with hexane-isoamyl alcohol (99.5:0.5 vol/vol). After centrifugation for 10 min at 10,000*g*, the organic phase was removed and quickly evaporated to dryness under a stream of nitrogen. The residue of the evaporation was redissolved in methanol-water (80:20 vol/vol), mixed by vortex, and a 20-μl aliquot was injected into the high-pressure liquid chromatography unit. This method has a lower level of detection at 50 ng/g tissue.

Experiment 2: Hemodynamics after Intrathecal Amitriptyline: Dose Response (Six Sheep)

A single distal port 18-gauge catheter (Portex) and left femoral arterial catheter were inserted and animals recovered from surgery and anesthesia, as described before. On the fourth postoperative day, a sling was positioned under the animals and arterial blood pressure and heart rate were monitored and recorded at 1-min intervals, as described previously. After 45 min of baseline recording, animals were randomized to receive either saline or amitriptyline (0.25, 1, or 5 mg) by slow intrathecal injection. Experiments were separated by at least 48 h.

Animals were observed for 2 h after intrathecal injection for behavioral changes or evidence of motor blockade, as described already. Arterial blood was withdrawn at baseline and 45 and 90 min after intrathecal injection for analysis of blood gas tension and *p*H.

Experiment 3: Antinociception and Behavior after Cervical Intrathecal Amitriptyline (Four Sheep)

A single distal port 18-gauge catheter (Portex) was inserted under direct vision *via* a small laminotomy at the second cervical interspace and advanced 4 cm caudad, and animals recovered from surgery and anesthesia as described already. On the fourth postoperative

day, antinociception before and after intrathecal injection of 5 mg amitriptyline was measured as threshold to a withdrawal response to pressure applied to the distal foreleg, as previously described.[16] Mechanical thresholds were measured using a device that gradually pushed a blunt pinhead against the skin over the lower end of the foreleg. The force applied to the pin was measured electronically and converted to Newtons (N) using calibration data. A cutoff pressure of 20 N was not exceeded to avoid tissue damage. Two days later, ewes received 10 mg amitriptyline according to the same protocol.

Drugs and Solutions. Ketamine, halothane, and penicillin were obtained from Barber Veterinary Supply (Richmond, VA), gentamicin from SoloPak Laboratories (Elk Grove, IL), and ampicillin from Apothecon (Princeton, NJ). Amitriptyline (Elavil) was purchased on the open market from Stuart Pharmaceutical Company (Wilmington, DE). This preparation contains 10 mg/ml amitripytline and the preservatives methylparaben (1.5 mg/ml) and propylparben (0.2 mg/ml) in sterile saline. Amitriptyline was diluted in sterile saline to a volume of 0.5 ml and injected for a period of 30 s, followed by injection of 0.5 ml saline (two times the catheter dead space).

Data Analysis. Although some data are presented as fractions of the baseline, all analyses were performed on raw data. Unless otherwise indicated, data are presented as means ± standard deviation. Effect of drug treatment and dose over time on spinal cord blood flow, hemodynamic variables, and arterial blood gas tensions were analyzed using a mixed-effect repeated-measures design with SAS System software (SAS Institute, Cary, NC) with adjustments for multiple comparisons by Fisher's protected LSD test. A *post hoc* analysis was performed to determine power to detect a decrease in spinal cord blood flow as great as 50%. Cerebrospinal fluid catecholamine and amitriptyline concentrations were compared between treatment and control groups and between near and distal tissues, respectively, using Student's *t* test. Probability values less than 0.05 were considered significant.

Results

Experiment 1: Spinal Cord Blood Flow and Hemodynamics after Intrathecal Amitriptyline

Low thoracic intrathecal injection of saline produced no behavioral changes, whereas injection of 5 mg amitriptyline produced a brief (<10 min) period of agitation without vocalization in 30% of animals, characterized by turning of the head and attempting to bite the catheters or the sling. This was followed in 30 min by mild to moderate sedation, with eyes closed and slumping in the sling. On testing, however, animals were easily aroused and there was no evidence of motor weakness or dysfunction in upper or lower extremities.

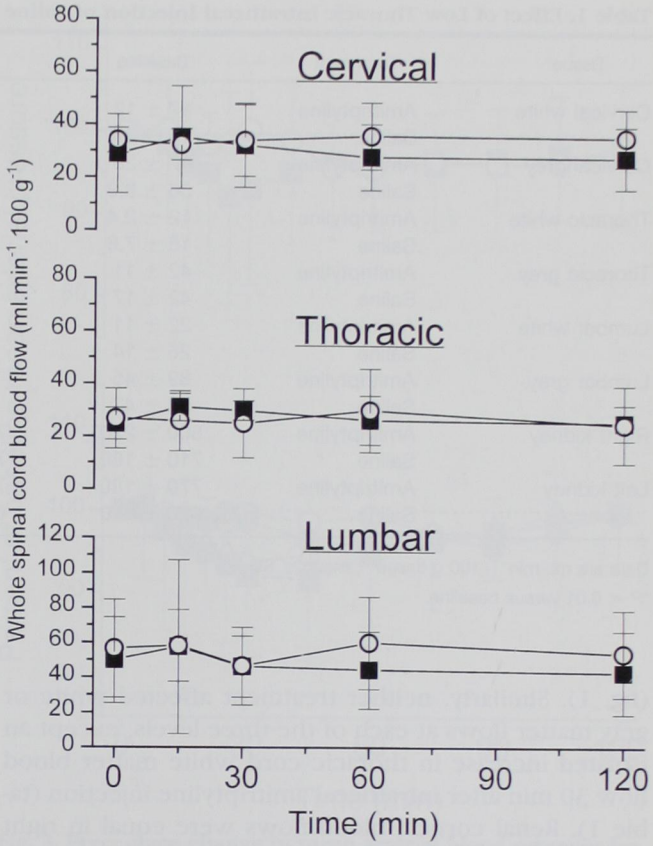

Fig. 1. Whole spinal cord blood flow before and after low thoracic intrathecal injection, at time 0, of saline (○) or 5 mg amitriptyline (■) in cervical, thoracic, or lumbar tissue. Data shown are means ± standard deviation. No significant differences between groups or within each group were found compared with baseline values.

Blood flow data from three animals were discarded due to technical errors. Baseline blood flow was higher in lumbar than in the thoracic or cervical cord (fig. 1). Baseline blood flow was higher in gray than in white matter at all levels of the cord, and both gray and white matter blood flow were higher at baseline in lumbar than in thoracic or cervical cord (table 1). Neither intrathecal saline nor amitriptyline affected total spinal cord blood flow in cervical, thoracic, or cervical cord

Table 1. Effect of Low Thoracic Intrathecal Injection of Saline or Amitriptyline on Spinal Cord Blood Flow

Tissue	Treatment	Baseline	+15 min	+30 min	+60 min	+120 min
Cervical white	Amitriptyline	17 ± 12	21 ± 21	22 ± 25	14 ± 7.4	11 ± 6.6
	Saline	14 ± 3.4	15 ± 6.5	13 ± 2.5	17 ± 6.0	12 ± 3.3
Cervical grey	Amitriptyline	47 ± 26	56 ± 40	47 ± 18	46 ± 22	49 ± 21
	Saline	56 ± 8.9	52 ± 11	55 ± 25	72 ± 27	57 ± 25
Thoracic white	Amitriptyline	12 ± 2.4	19 ± 4.0	19 ± 6.1*	15 ± 4.7	24 ± 29
	Saline	15 ± 7.6	16 ± 6.3	12 ± 5.1	15 ± 7.8	12 ± 5.8
Thoracic grey	Amitriptyline	42 ± 11	46 ± 10	44 ± 9.2	41 ± 17	50 ± 32
	Saline	42 ± 17	39 ± 18	42 ± 21	47 ± 22	37 ± 20
Lumbar white	Amitriptyline	22 ± 11	32 ± 29	28 ± 12	27 ± 18	23 ± 12
	Saline	26 ± 14	25 ± 9.6	22 ± 6.0	29 ± 7.8	25 ± 8.2
Lumbar grey	Amitriptyline	89 ± 45	93 ± 69	47 ± 29	71 ± 32	72 ± 40
	Saline	93 ± 47	79 ± 22	75 ± 40	95 ± 45	87 ± 40
Right kidney	Amitriptyline	800 ± 220	770 ± 210	750 ± 190	800 ± 240	730 ± 180
	Saline	710 ± 180	700 ± 190	650 ± 200	700 ± 230	630 ± 140
Left kidney	Amitriptyline	770 ± 180	750 ± 170	750 ± 190	750 ± 170	700 ± 170
	Saline	680 ± 170	690 ± 160	620 ± 210	630 ± 250	590 ± 170

Data are ml · min^{-1} · 100 g tissue^{-1}; mean ± SD.

*$P < 0.01$ *versus* baseline.

(fig. 1). Similarly, neither treatment affected white or gray matter flows at each of the three levels, except an isolated increase in thoracic cord white matter blood flow 30 min after intrathecal amitriptyline injection (table 1). Renal cortical blood flows were equal in right and left kidneys and were unaffected by intrathecal drug injection (table 1).

Figure 2, which depicts individual animal data from lumbar cord, shows the marked inter-animal variability in spinal cord blood flow. Similar inter-animal variability existed for thoracic and cervical cord as well. Despite this variability, there was no clear trend toward an increase or decrease in spinal cord blood flow in amitriptyline-treated animals, nor were there individual cases of large increases or decreases in spinal cord blood flow that may have been lost in the variability (fig. 2). Despite the large inter-animal variability, power analysis revealed a power of 80% to detect a clinically significant 50% decrease in spinal cord blood flow, and power of 55% to detect a 25% decrease in spinal cord blood flow.

Except for cardiac output, which was greater in animals receiving amitriptyline, the groups did not differ in hemodynamic variables before intrathecal injection. Neither treatment affected cardiac output or pulmonary arterial pressure, although both were associated with statistically significant but minor decreases in central venous pressure (table 2).

Groups did not differ in mean arterial blood pressure (94 ± 11 mmHg for saline, 95 ± 10 mmHg for amitriptyline) or in heart rate (104 ± 11 beats/min for saline, 109 ± 15 beats/min for amitriptyline) before intrathecal injection. Neither intrathecal saline nor amitriptyline injection affected mean arterial blood pressure (fig. 3A) or heart rate.

Amitriptyline concentration was greater in spinal cord gray matter near the site of injection (5,880 ± 2,650 ng/g tissue) than in white matter at this site (2,870 ± 1,510 ng/g tissue), and both sites had a greater amitriptyline concentration than was present in CSF (1,320 ± 630 ng/ml). Amitriptyline concentrations were more than two orders of magnitude greater in spinal cord tissue near the site of injection than in the cervical spinal cord (22 ± 11 ng/g tissue in gray matter and 17 ± 10 ng/g tissue in white matter). Groups did not differ in arterial *p*H, partial pressure of oxygen or carbon dioxide (data not shown), or in CSF norepinephrine, epinephrine, and dopamine concentrations at the end of the experiment (saline-treated animals had 26 ± 18 nmol/ml norepinephrine, 10 ± 4.9 epinephrine, and 16 ± 20 nmol/ml dopamine, whereas amitriptyline-treated animals had 30 ± 24 nmol/ml norepinephrine, 15 ± 21 nmol/ml epinephrine, and 16 ± 15 nmol/ml dopamine in CSF).

Experiment 2: Hemodynamics after Thoracic Intrathecal Amitriptyline: Dose Response

Intrathecal injection of saline or 0.25 mg amitriptyline did not alter animal behavior. Three of six ewes exhib-

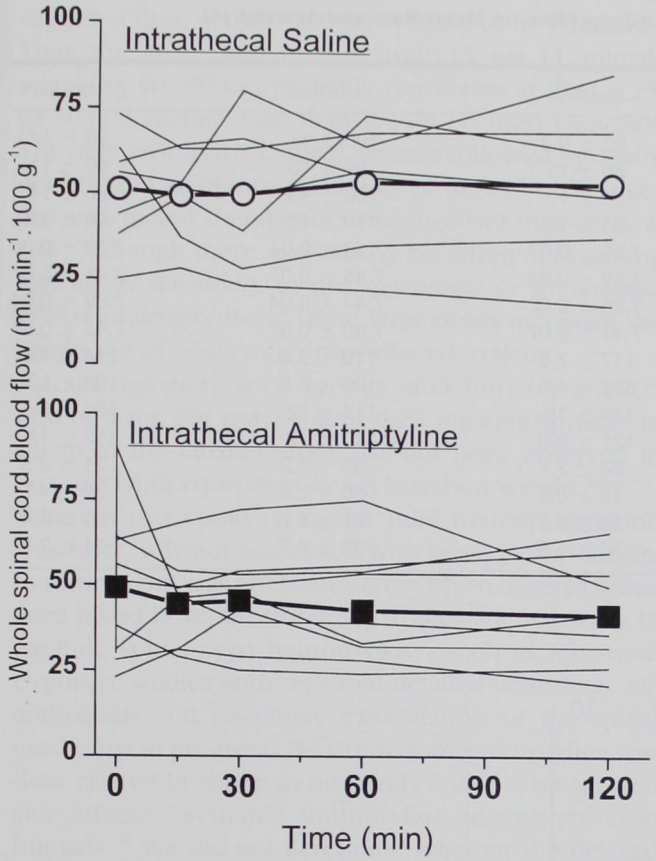

Fig. 2. Whole lumbar spinal cord blood flow before and after low thoracic intrathecal injection, at time 0, of saline (○) or 5 mg amitriptyline (■). Individual animal data are shown in thin lines, and averages are shown as the thick line with circles. No significant differences between groups or within each group were found compared with baseline values.

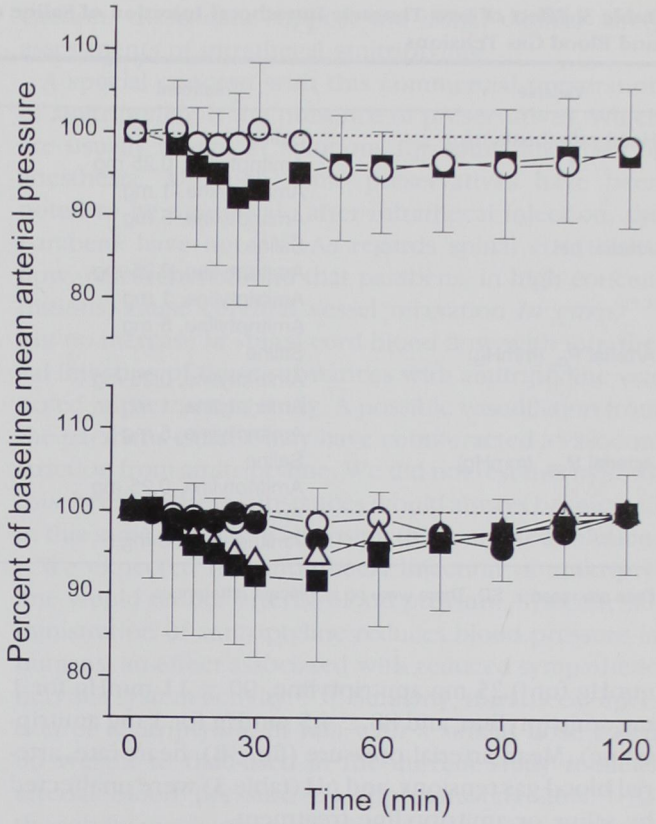

Fig. 3. Percentage change in mean arterial blood pressure before and after low thoracic injection, at time 0, of saline (09) or 5 mg amitriptyline (■) in the spinal cord blood flow study or of saline (○) or 0.25 mg amitriptyline (♦), 1 mg amitriptyline (△), or 5 mg amitriptyline (■) in the dose-response study. Data shown are means ± standard deviation. No significant differences were found among groups.

ited transient agitation as described in the spinal cord blood flow study after injection of 5 mg amitriptyline, whereas no such agitation was observed with 1 mg amitriptyline. Mild to moderate sedation was noted in animals receiving 1 or 5 mg amitriptyline, although animals were easily aroused and on testing had no evidence of motor weakness.

Groups did not differ in mean arterial blood pressure before injection (91 ± 6.7 mmHg for saline, 96 ± 25

Table 2. Effect of Low Thoracic Intrathecal Injection of Saline or Amitriptyline on Cardiac Output, Pulmonary Arterial Pressure, and Central Venous Pressure

Variable	Treatment	Baseline	+15 min	+30 min	+60 min	+120 min
Cardiac output (L/min)	Amitriptyline	7.3 ± 1.1	6.7 ± 1.6	6.7 ± 1.9	6.5 ± 1.9	6.6 ± 1.9
	Saline	6.1 ± 1.6	5.9 ± 1.6	5.9 ± 1.6	5.8 ± 1.6	5.5 ± 0.9
Mean pulmonary arterial pressure (mmHg)	Amitriptyline	23 ± 5.0	23 ± 5.3	21 ± 5.3	22 ± 5.3	20 ± 5.6
	Saline	24 ± 2.0	22 ± 3.6	22 ± 7.2	23 ± 3.6	24 ± 2.9
Central venous pressure (mmHg)	Amitriptyline	14 ± 13	11 ± 13	11 ± 13	7.9 ± 11	7.8 ± 9.5*
	Saline	16 ± 9.8	15 ± 7.6	14 ± 6.7	12 ± 6.9	9.8 ± 6.3*

Values are mean ± SD.

*$P < 0.05$ *versus* baseline.

Table 3. Effect of Low Thoracic Intrathecal Injection of Saline or Amitriptyline on Heart Rate and Arterial pH and Blood Gas Tensions

Variable	Treatment	Baseline	+45 min	+90 min
Heart rate (beats/min)	Saline	92 ± 9.8	100 ± 11	96 ± 17
	Amitriptyline, 0.25 mg	88 ± 15	99 ± 13	90 ± 11
	Amitriptyline, 1 mg	100 ± 10	100 ± 9.8	98 ± 14
	Amitriptyline, 5 mg	93 ± 13	110 ± 15	99 ± 7.4
Arterial *p*H	Saline	7.39 ± 0.07	7.42 ± 0.04	7.39 ± 0.04
	Amitriptyline, 0.25 mg	7.42 ± 0.04	7.45 ± 0.07	7.41 ± 0.07
	Amitriptyline, 1 mg	7.39 ± 0.02	7.44 ± 0.04	7.39 ± 0.04
	Amitriptyline, 5 mg	7.41 ± 0.07	7.40 ± 0.07	7.43 ± 0.07
Arterial P_{O_2} (mmHg)	Saline	117 ± 7.6	119 ± 3.8	114 ± 7.6
	Amitriptyline, 0.25 mg	115 ± 4.0	109 ± 9.8	103 ± 10
	Amitriptyline, 1 mg	114 ± 16	114 ± 12	104 ± 8.5
	Amitriptyline, 5 mg	121 ± 8.0	110 ± 12	112 ± 12
Arterial P_{CO_2} (mmHg)	Saline	34 ± 2.7	32 ± 2.2	31 ± 2.5
	Amitriptyline, 0.25 mg	29 ± 6.5	30 ± 7.4	32 ± 4.7
	Amitriptyline, 1 mg	34 ± 6.5	27 ± 4.7	31 ± 3.4
	Amitriptyline, 5 mg	30 ± 5.8	33 ± 4.7	31 ± 2.5

Data are mean ± SD. There were no significant differences.

mmHg for 0.25 mg amitriptyline, 90 ± 11 mmHg for 1 mg amitriptyline, and 88 ± 4.5 mmHg for 1 mg amitriptyline). Mean arterial pressure (fig. 3B), heart rate, arterial blood gas tensions, and *p*H (table 3) were unaffected by saline or amitriptyline treatment.

Experiment 3: Antinociception and Behavior after Cervical Intrathecal Amitriptyline

Cervical intrathecal injection of 5 mg amitriptyline produced antinociception to mechanical stimulation that was brief and mild, representing less than 60% of the maximal cutoff value of 20 N (fig. 4). Animals were sedated, beginning 10 min after injection and lasting 30–60 min thereafter. Intrathecal injection of 10 mg amitriptyline produced intense sedation in animals, manifested by minimal or no response to noise or antinociception testing. One of the four animals had a generalized seizure 5 min after injection of 10 mg amitriptyline and died. Because arterial catheters had not been inserted in these animals, we could not determine whether these dysrhythmias led to death.

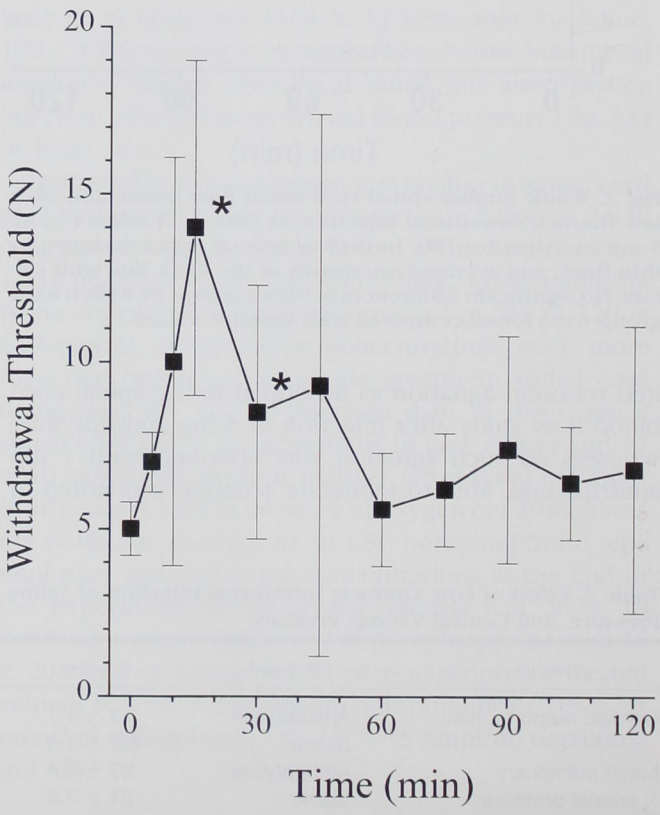

Fig. 4. Antinociception, shown as threshold to withdrawal from a mechanical stimulus to the forelimb, is expressed in Newtons (N), before and after cervical intrathecal injection, at time 0, of 5 mg amitriptyline. Data are shown as means ± standard deviation. *$P < 0.05$ compared with baseline values.

Discussion

Before studies can be done in humans, preclinical toxicity screening of novel agents for spinal injection is mandatory. Although the therapeutic dose of amitriptyline in humans is unknown, extrapolation of data obtained in rats with amitriptyline and other analgesics

suggest a dose of 100–200 μg is likely to be effective.[1] Thus the dose used in this study (5 mg in animals weighing 40–50 kg) probably represents at least a 25- to 50-fold higher dose than would be used clinically. The only serious toxic effect, seizure followed by death, occurred in an animal receiving 10 mg (50–100 times the anticipated therapeutic human dose) into cervical CSF. Although there may clearly be differences among species in sensitivity to pharmacologic or toxicologic effects of drugs, use of these large doses increases the likelihood of observing drug-induced toxicity.

Acute agitation, such as seen after intrathecal injection of 5 mg but not 1 mg or 0.25 mg amitriptyline in sheep in the current study has not been observed in rats receiving equivalent doses based on weight.[1,2,17–19] Whereas this could represent mild toxicity, agitation was brief, was not associated with gross motor deficits to testing, and was not associated with changes in spinal cord blood flow. Clearly other studies are necessary to exclude spinal cord neurotoxicity, such as long-term exposure studies with repeated detailed neurologic examinations and histologic examination of the spinal cord (now in progress). Sedation from amitriptyline was dose related in sheep in our study and is a recognized side effect of systemic amitriptyline administration in humans.[20] We did not determine whether the delayed onset of this sedation was a result of rostral spread in CSF or systemic absorption. Seizures are a recognized side effect of oral amitriptyline overdose[21] and were not surprising after a large dose administered into cervical CSF in sheep in the current study.

Intrathecal amitriptyline injection did not reduce spinal cord blood flow. Several aspects of the study design strengthen the conclusion that spinal cord blood flow is not reduced by intrathecal amitriptyline in sheep. First, the dose used was large and had clear pharmacologic effects (antinociception and sedation). Second, despite large variability among animals in blood flow, examination of individual animal data did not reveal large decreases in blood flow, and the power of the study was excellent to exclude a clinically significant reduction in blood flow. Third, examination of several regions of spinal cord, both near and far from the site of injection, failed to detect a difference in change in blood flow after injection, despite large differences in tissue concentrations of drug. Finally, separate analysis of gray and white matter revealed no evidence of reduction in either compartment. Whereas data obtained in animals should be extrapolated to humans only with caution, these data support the initial clinical safety assessments of intrathecal amitriptyline.

A special concern with this commercial preparation of amitriptyline is the presence of preservatives, which are usually absent in solutions for spinal analgesia or anesthesia. Although some preservatives have been noted to be neurotoxic after intrathecal injection, the parabens have not.[22–24] As regards spinal cord blood flow, researchers found that parabens, in high concentrations, cause cerebral vessel relaxation *in vitro*,[25,26] but no increase in spinal cord blood flow with intrathecal injection of these substances with amitriptyline was noted in the current study. A possible vasodilation from the parabens exactly may have counteracted a vasoconstriction from amitriptyline. We did not test this hypothesis, because these substances would always be injected in this same ratio clinically using this marketed solution.

We expected that intrathecal injection of amitriptyline would reduce arterial blood pressure. Systemic administration of amitriptyline reduces blood pressure in humans, an effect associated with reduced sympathetic nervous system activity.[27–29] Similarly, intrathecal injection of amitriptyline in rats with a similar dose based on weight to that used in the current study reduced arterial blood pressure by approximately 20%.[18] Although its mechanism of action is unknown, amitriptyline could reduce blood pressure after intrathecal administration by enhancing noradrenergic input or by producing muscarinic blockade on preganglionic sympathetic neurons, because either effect should reduce sympathetic activity.[30,31] However, only mild, nonsignificant decreases (< 10%) were observed after injection of the largest dose (5 mg) in the current study in sheep. This could represent species difference, because intrathecal injection of the α_2-adrenergic agonist clonidine produces mild to no reductions in arterial blood pressure in sheep,[32,33] although larger decreases are found in humans.[34] Alternatively, this could relate to the larger size of the spinal cord in sheep and humans than in rats, a proposed explanation for the rapid and large increase in arterial blood pressure after intrathecal administration of neostigmine in rats[35] but minimal or no effect in sheep[36] or humans.[37,38] Thus these results do not preclude decreased arterial blood pressure as a side effect from intrathecal amitriptyline administration in humans.

Although the current study was not designed to provide detailed pharmacokinetics, it does provide the first data regarding amitriptyline distribution after intrathecal administration. The 100-fold larger tissue concentra-

tion of drug in spinal cord tissue near the site of injection compared with that in cervical cord 2 h after injection is consistent with similar observations after lumbar intrathecal injection of other lipophilic and hydrophilic agents.[39,40] Larger concentrations of amitriptyline in spinal cord tissue than in CSF near the injection site are consistent with partitioning into a lipid-rich environment. Larger concentrations in spinal cord gray matter than white matter 2 h after injection suggest an efficient movement of drug to sites of potential analgesic action.

Finally, other investigators have proposed that enhancement of analgesia from systemically administered opioids by intrathecal injection of amitriptyline and other antidepressants is due to activation by opioids of descending spinal noradrenergic transmission and inhibition monoamine reuptake by the antidepressant.[17,19,41] We could not indirectly confirm this hypothesis, because intrathecal amitriptyline did not increase CSF concentrations of norepinephrine. However, because intrathecal amitriptyline alone produces minimal to no analgesia in healthy rats[1] and sheep (current study), it is conceivable that there is little tonic activity of these descending noradrenergic pathways in the normal state and that inhibition of reuptake should have little effect on synaptic or CSF concentrations of norepinephrine. Alternatively CSF concentrations may not actively reflect synaptic concentrations of norepinephrine, although similar increases in CSF and dorsal horn microdialysate concentrations of norepinephrine are observed after intravenous morphine administration in sheep.[42]

Intrathecal amitriptyline produces a brief period of agitation but does not affect spinal cord blood flow or arterial blood pressure in sheep, and CSF and spinal cord tissue concentrations of amitriptyline are consistent with efficient penetration of the spinal cord and rostral distribution over time. It is conceivable that rostral spread of amitriptyline in CSF could produce seizures, although this was only observed in one of four sheep receiving a large dose of amitriptyline (10 mg) injected into cervical CSF. These data suggest that intrathecal amitriptyline injection, in doses much greater than those anticipated for clinical use, do not produce worrisome changes in either blood pressure or spinal cord blood flow. Eventual clinical trials await further toxicity testing.

The authors thank Barbara Tucker for help in animal preparation and Ellen Tommasi for performing the catecholamine assays.

References

1. Eisenach JC, Gebhart GF: Intrathecal amitriptyline - antiociceptive interactions with intravenous morphine and intrathecal clonidine, neostigmine, and carbamylcholine in rats. ANESTHESIOLOGY 1995; 83:1036 - 45

2. Eisenach JC, Gebhart GF: Intrathecal amitriptyline acts as an N-methyl-D-asparate receptor antagonist in the presence of inflammatory hyperalgesia in rats. ANESTHESIOLOGY 1995; 83:1046 - 54

3. Fields HL, Heinricher MM, Mason P: Neurotransmitters in nociceptive modulatory circuits. Annu Rev Neurosci 1991; 14:219 - 45

4. Haley JE, Sullivan AF, Dickenson AH: Evidence for spinal *N*-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. Brain Res 1990; 518:218 - 26

5. Davar G, Hama A, Deykin A, Vos B, Maciewicz R: MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy. Brain Res 1991; 553:327 - 30

6. Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, Willis WD: Neurogenic hyperalgesia: central neural correlates in responses of spinothalamic tract neurons. J Neurophysiol 1991; 66:228 - 46

7. Sernagor E, Kuhn D, Vyklicky L Jr, Mayer ML: Open channel block of NMDA receptor responses evoked by tricyclic antidepressants. Neuron 1989; 2:1221 - 7

8. Cai Z, McCaslin PP: Amitriptyline, desipramine, cyproheptadine and carbamazepine, in concentrations used therapeutically, reduce kainate- and N-methyl-D-aspartate-induced intracellular Ca^{2+} levels in neuronal culture. Eur J Pharmacol 1992; 219:53 - 7

9. Reynolds IJ, Miller RJ: Tricyclic antidepressants block N-methyl-D-aspartate receptors: similarities to the action of zinc. Br J Pharmacol 1988; 95:95 - 102

10. Yaksh TL, Collins JG: Studies in animals should precede human use of spinally administered drugs. ANESTHESIOLOGY 1989; 70:4 - 6

11. Collins JG: Spinally administered neostigmine — Something to celebrate. ANESTHESIOLOGY 1995; 82:327 - 8

12. Hale SL, Alker KJ, Kloner RA: Evaluation of nonradioactive, colored microspheres for measurement of regional myocardial blood flow in dogs. Circulation 1988; 78:428 - 34

13. Eisenach JC, Tong C, Limauro D: Intrathecal clonidine and the response to hemorrhage. ANESTHESIOLOGY 1992; 77:522 - 8.

14. Coudore F, Ardid D, Eschalier A, Lavarenne J, Fialip J: High-performance liquid chromatographic determination of amitriptyline and its main metabolites using a silica column with reversed-phase eluent. Application in mice. J Chromatogr B Biomed Appl 1992; 584:249 - 55

15. Coudore F, Ardid D, Eschalier A, Lavarenne J: High-performance liquid chromatographic determination of amitriptyline and its main metabolites using a silica column with reversed-phase eluent. J Chromatography 1992; 584:249 - 55

16. Nolan A, Livingston A, Morris R, Waterman A: Techniques for comparison of thermal and mechanical nociceptive stimuli in the sheep. J Pharmacol Meth 1987; 17:39 - 49

17. Botney M, Fields HL: Amitriptyline potentiates morphine analgesia by a direct action on the central nervous system. Ann Neurol 1983; 13:160 - 4

18. Dirksen R, Van Diejen D, Van Luijtelaar ELJM, Booij LHDJ: Site- and test-dependent antinociceptive efficacy of amitriptyline in rats. Pharmacol Biochem Behav 1994; 47:21 - 6

19. Larsen J-J, Arnt J: Spinal 5-HT or NA uptake inhibition potenti-

ates supraspinal morphine antinociception in rats. Acta Pharmacol Toxicol 1984; 54:72–5

20. Onghena P, Houdenhove BV: Antidepressant-induced analgesia in chronic non-malignant pain: a meta-analysis of 39 placebo-controlled studies. Pain 1992; 49:205–19

21. Hultén B-Å, Heath A, Knudsen K, Nyberg G, Starmark J-E, Martensson E: Severe amitriptyline overdose: relationship between toxicokinetics and toxicodynamics. Clin Toxicol 1992; 30:171–9

22. Rowlingson JC: Toxicity of local anesthetic additives. Reg Anesth 1993; 18:453–60

23. Adams HJ, Mastri AR, Charron D: Morphological effects of subarachnoid methylparaben on rabbit spinal cord. Pharmacol Res Comm 1977; 9:547–51

24. Mizuno K, Ogawa S, Itoh S: Supressive effect of methylparaben on the evoked compound action potentials in excised rabbit cervical vagus nerve. Matsui-Japanese Journal of Anesthesiology 1994; 43:1008–14

25. Hamilton JT, Zhou Y, Gelb AW: Paraben preservatives but not succinylcholine are cerebral vasodilators in vitro. ANESTHESIOLOGY 1990; 73:1252–7

26. Brandt L, Andersson KE, Hindfelt B, Ljunggren B, Pickard JD: Are the vascular effects of naloxone attributable to the preservatives methyl- and propylparaben? Journal of Cerebral Blood Flow and Metabolism 1983; 3:395–8

27. Flett SR, Szabadi E, Bradshaw CM: A comparison of the effects of fluvoxamine and amitriptyline on autonomic functions in healthy volunteers. Eur J Clin Pharmacol 1992; 42:529–33

28. Low PA, Opfer-Gehrking TL: Differential effects of amitriptyline on sudomotor, cardiovagal, and adrenergic function in human subjects. Muscle Nerve 1992; 15:1340–4

29. Bourne M, Szabadi E, Bradshaw CM: A comparison of the effects of single doses of amoxapine and amitriptyline on autonomic functions in healthy volunteers. Eur J Clin Pharmacol 1993; 44:57–62

30. Ryall RW: Effect of monoamines upon sympathetic preganglionic neurons. Circ Res 1967; 20-21(Suppl III):III-83–87

31. Bhargava KP, Pant KK, Tangri KK: Cholinergic influences on the spinal cardiovascular neurones. J Auton Pharmacol 1982; 2:225–30

32. Eisenach JC, Dewan DM, Rose JC, Angelo JM: Epidural clonidine produces antinociception, but not hypotension, in sheep. ANESTHESIOLOGY 1987; 66:496–501

33. Eisenach JC, Tong C: Site of hemodynamic effects of intrathecal α_2-adrenergic agonists. ANESTHESIOLOGY 1991; 74:766–771

34. Coombs DW, Saunders RL, LaChance D, Savage S, Ragnarsson TS, Jensen LE: Intrathecal morphine tolerance: Use of intrathecal clonidine, DADLE, and intraventricular morphine. ANESTHESIOLOGY 1985; 62:357–63

35. Magrí V, Buccafusco JJ: Hypertension following intrathecal injection of cholinergic agonists in conscious rats: role of endogenous acetylcholine. J Auton Nerv Syst 1988; 25:69–77

36. Williams JS, Tong C, Eisenach JC: Neostigmine counteracts spinal clonidine-induced hypotension in sheep. ANESTHESIOLOGY 1993; 78:301–7

37. Hood DD, Eisenach JC, Tuttle R: Phase I safety assessment of intrathecal neostigmine in humans. ANESTHESIOLOGY 1995; 82:331–43

38. Lauretti GR, Lima ICPR: The effects of intrathecal neostigmine on somatic and visceral pain: improvement by association with a peripheral anticholinergic. Anesth Analg 1996; 82:617–20

39. Post C, Gordh T Jr, Minor BG, Archer T, Freedman J: Antinociceptive effects and spinal cord tissue concentrations after intrathecal injection of guanfacine or clonidine into rats. Anesth Analg 1987; 66:317–24

40. Gustafsson LL, Post C, Edvardsen B, Ramsay CH: Distribution of morphine and meperidine after intrathecal administration in rat and mouse. ANESTHESIOLOGY 1985; 63:483—9

41. Taiwo YO, Fabian A, Pazoles CJ, Fields HL: Potentiation of morphine antinociception by monoamine reuptake inhibitors in the rat spinal cord. Pain 1985; 21:329–37

42. Bouaziz H, Tong CY, Yoon Y, Hood DD, Eisenach JC: Intravenous opioids stimulate norepinephrine and acetylcholine release in spinal cord dorsal horn — systematic studies in sheep and an observation in a human. ANESTHESIOLOGY 1996; 84:143–54