Objective—To evaluate perineal analgesic effects of 3 doses of neostigmine coadministered epidurally with lidocaine to geldings.

Animals—6 healthy geldings.

Procedures—A few days before each treatment, a catheter was inserted between the first and second coccygeal vertebrae via the caudal approach in each gelding; the catheter tip was threaded approximately 10 cm cranial into the midsacral region. Each horse received 4 epidural treatments: 2% lidocaine (0.2 mg/kg) alone and 3 doses of neostigmine (0.5, 1, or 2 µg/kg) coadministered with that same dose of lidocaine. Horses were restrained in stocks in a standing position. Heart rate, blood pressure, respiratory rate, rectal temperature, intestinal motility, analgesia, behavior, and ataxia were determined before treatment (time 0; baseline); at 5, 10, 15, 30, 45, 60, 75, and 90 minutes; and every 30 minutes thereafter until the cessation of analgesia.

Results—All doses of neostigmine coadministered with lidocaine improved and extended the duration of analgesia in the perineal region of the geldings. Total duration of analgesia was not a dose-dependent effect (120, 150, and 150 minutes for 0.5, 1, and 2 µg/kg, respectively). All treatments induced mild or moderate ataxia. Cardiovascular changes were within acceptable limits.

Conclusions and Clinical Relevance—Administration of neostigmine (1 µg/kg) combined with lidocaine (0.2 mg/kg) in the caudal epidural space induced analgesia for 2.5 hours with a low prevalence of adverse effects in standing conscious geldings. Epidural doses of neostigmine greater than these should be avoided because they may cause undesirable effects in geldings. (Am J Vet Res 2012;73:1356–1362)

A major complication of local anesthesia via epidural administration is the indiscriminate blockade of sensory, motor, and sympathetic fibers. In addition, a short duration of analgesia is among the limitations of local anesthesia via a single caudal epidural injection.1 A blockade of pain by drugs that act at the spinal level without concurrent motor and sympathetic blockade is ideal to allow ambulation of horses and to avoid cardiovascular alterations or convulsions.

To provide better control for pain after surgery or injury and to maintain such control long term in humans, treatment has advanced from epidural administration of a single drug to coadministration of 2 or 3 drugs. Opioids are often used as adjuvants to relieve signs of pain in horses.5,6 Other options, including α2-adrenergic receptor agonists7–9 or N-methyl-D-aspartate receptor antagonists,10,11,12 for induction of analgesia via epidural administration have been used in horses.

Cholinesterase inhibitors, which inhibit the breakdown of endogenous acetylcholine and thus indirectly stimulate both muscarinic and nicotinic receptors, have been used to induce spinal analgesia in sheep,13 dogs,14,15 and humans.3,14,15 Neostigmine is a cholinomimetic agent used to antagonize the action of nondepolarizing neuromuscular blocking agents.13,14 Investigators in 1 study16 determined that analgesia induced by acetylcholine and synthetic cholinergic receptor agonists is inhibited by muscarinic but not nicotinic receptor antagonists, which indicates an action on muscarinic receptors. Intrathecal administration of neostigmine in humans induces dose-dependent analgesia. However, the analgesia is associated with a high incidence of severe adverse effects, such as vomiting, diarrhea, and intense nausea; thus, the routine clinical use of neo-
stigmine is limited. To our knowledge, analgesia and adverse effects after epidural administration of neostigmine have not been investigated in horses. The purpose of the study reported here was to evaluate analgesia and adverse effects after caudal epidural coadministrition of neostigmine and lidocaine in healthy conscious geldings.

Materials and Methods

Animals—Six healthy geldings that weighed between 400 and 460 kg (mean ± SD, 433 ± 21 kg) and that were 7 to 13 years old (mean age, 13 ± 3 years) were selected for use in the study. Prior to the experiments, the geldings were moved into stalls in the Faculty of Veterinary Medicine and Animal Science at the Federal University of Mato Grosso do Sul; horses remained in these stalls and had ad libitum access to hay and water throughout the experimental period. A routine clinical examination was performed to ensure that the horses were healthy. The study protocol was approved by the Federal University of Mato Grosso do Sul State Animal Care and Use Committee.

Experimental preparation—A few days before each treatment, a catheter was inserted in the caudal epidural space of each horse to reduce risks associated with repeated epidural injections. For the insertion of the catheters, each gelding was restrained in stocks and sedated with 1% acepromazine maleate (0.1 mg/kg, IV). The first intercoccygeal space was identified by manipulating the tail in dorsal and ventral directions during simultaneous palpation of the depression between the first and second coccygeal vertebrae. The skin overlying the area was aseptically prepared with povidone iodine and infiltrated with 2% lidocaine at the entry point. A small incision (approx 0.8 cm) was made in the skin and subcutaneous tissue of this area. An 18-gauge Tuohy needle and 18-gauge epidural catheter were used for caudal epidural catheterization and subsequent drug administration. Correct needle placement was confirmed by the hanging-drop method and a lack of palpable resistance during catheter insertion. The catheter was threaded 10 cm cranially to the mid-sacral region (approximately between S2 and S3). The catheter was cut (total length, 30 cm) and connected to a valve. The catheter was affixed to the skin with cyanoacrylate glue and wrapped in gauze. At the end of each experiment, the catheters were removed and evaluated for gross evidence of infection and proper insertion. The catheters were then submitted for bacterial culture and submersion in blood agar for detection of possible contamination.

Procedures—During the week after catheter placement, the horses were trained to stand quietly in stocks for periods of several hours. Each horse received 4 treatments. Treatments were 2% lidocaine (0.2 mg/kg; mean dose, 4.1 mL) without epinephrine and that same dose of lidocaine combined with neostigmine methylsulfate at each of 3 doses (0.5 µg/kg [mean dose, 4.6 mL], 1 µg/kg [mean dose, 5.0 mL], and 2 µg/kg [mean dose, 5.9 mL]). The order of treatments for each horse was chosen at random; there was a period of at least 1 week between subsequent treatments. Drugs were administered at a rate of 0.5 mL/s through the catheter. After drug administration, catheters were filled with saline (0.9% NaCl) solution. To minimize differences in ambient temperature between treatments, all experiments were conducted in the morning, with a target temperature of 25°C.

Analgesia assessment—Heart rate, respiratory rate, SAP, DAP, MAP, rectal temperature, intestinal movements, analgesia, behavior, and motor blockade were determined before drug administration (time 0); at 5, 10, 15, 30, 45, 60, 75, and 90 minutes; and every 30 minutes thereafter until a score < 3 was achieved for the pain scoring system (scale of 1 to 4; Appendix). Two analgesia tests were used (pinprick [deep analgesia] and thermal [superficial analgesia] noxious stimuli), and lack of analgesia (a strong positive response to both noxious stimuli) was verified at time 0. Evaluators were not aware of the treatment administered to each horse.

First, all horses received a standard noxious stimulus consisting of skin and muscle pinprick of the perineum (perineal dermatome innervated by spinal nerve S3 and S4), tail (tail dermatome innervated by spinal nerves originating from the coccyx to spinal nerve S3), and dorsal aspect of the hind limb (dorsal hind limb dermatomes innervated by spinal nerves originating from the coccyx to spinal nerve S2) with a 22-gauge, 2.5-cm-long needle (Figure 1).

The second analgesic test was thermal stimulation with a water-filled tube that was maintained at a constant temperature (65°C) in a thermostatically controlled water bath. Thermal stimulation testing was performed bilaterally at the same locations as the pinpricks by lightly applying the tube tip to the skin for a maximum time of 30 seconds. For each point evaluated, different tubes were used to avoid differences in temperature attributable to cooling. Thermal pain response was evaluated, and the stimulation time was measured with a stopwatch. Movements of head, limbs, and tail; attempts to kick; and turning of the head toward the stimulation site were considered a positive pain response, and the tube tip was immediately removed from contact with the skin. Thermal stimulation testing was performed by the same researcher (FBM) throughout the experimental period.

Horses were evaluated for motor blockade or presence of ataxia by walking them out of the stocks at 15-minute intervals from 15 to 60 minutes and at 30-minute intervals thereafter until the end of each experiment. Scoring of intestinal motility was conducted via auscultation of 1 quadrant (dorsal aspect of the right flank) for 1 minute. Intestinal motility was scored on a scale of 0 to 5, where 0, 1, 2, 3, 4, and 5 represented 0, 1, 2, 3, 4, and ≥ 5 sounds/min, respectively. Analgesia, behavior, and motor blockade were evaluated by use of a descriptive scale (Appendix).

Baseline values for clinical variables were recorded before epidural administration of the drugs (time 0). Arterial pressures were measured with a cardiac monitor by means of a noninvasive oscillometric device with the cuff placed over the coccygeal artery. Heart rate was measured via the cardiac monitor and recorded as the number of beats per minute. Respiratory rate was the...
number of chest movements per minute. Rectal temperature was measured with a digital thermometer. Responses to noise or sudden movements of personnel were also recorded.

**Statistical analysis**—All data were analyzed by use of a commercial software program. Data were summarized as mean ± SEM. A randomized block design was used for each drug; in this case, each horse was considered a block and each time considered a treatment. For the dependent variables SAP, DAP, MAP, heart rate, respiratory rate, and rectal temperature, an ANOVA was used to determine whether there were significant differences from the values at time 0. For analgesia, behavior, and motor blockade variables, the nonparametric Friedman test was used, followed by multiple comparisons. The Dunnett rank test was also used, with time 0 being considered as baseline. In each analysis, differences were considered significant at values of $P < 0.05$.

**Results**

Analgesia was induced in the tail, perineum, and dorsal aspect of the hind limbs in all horses after caudal epidural administration of each treatment. Placement of all epidural catheters was performed without problems, and no contamination was observed on the catheters at the time of removal. Epidural administration of the 3 doses of neostigmine did not result in adverse behavioral changes, sedation, or recumbency in any of the horses in the study. None of the 4 treatments induced any adverse behavioral changes (eg, deep sedation or excitation). No horses developed signs of ileus or colic during the experimental period or during the week after treatment. On the basis of auscultation of 1 abdominal quadrant, none of the horses had intestinal immotility (score 0) or a substantial increase in intestinal motility (score 5).

After epidural administration of the 4 treatments, muscle tone of the tail became undetectable almost immediately (score 2). All horses developed moderate ataxia of the hind limbs (score 3; Table 1) 5 to 10 minutes after injection. The ataxic effect was more prolonged (120 minutes) with the neostigmine treatments, whereas the lidocaine treatment induced a shorter period of ataxia (approx 70 minutes).

Response to pinprick noxious stimulation (deep analgesia) and thermal stimulation (superficial analgesia) applied to the dermatome regions satisfactorily indicated the efficacy and extent of analgesia in the horses. Before the beginning of the experiments (time 0), all horses responded to the thermal stimulus with a mean ± SD response time of 8 ± 4 seconds at all stimulation points. The 4 epidural treatments significantly increased the mean response time to thermal stimulation (23 ± 7 seconds). The 3 treatments with neostigmine induced a longer duration of analgesia than did lidocaine alone. Mean ± SD duration of analgesia for the 3 neostigmine treatments (0.5 µg/kg [120 ± 15 minutes], 1 µg/kg [150 ± 35 minutes], and 2 µg/kg [150 ± 20 minutes]) differed significantly from that for the lidocaine treatment (70 ± 12 minutes; Table 1). However, no significant difference was detected among the 3 treatments with neostigmine. In the treatment with

![Figure 1—Schematic depiction of the caudal view of a horse indicating dermatomes that could be affected by drugs administered via the caudal epidural route to a horse maintained in a standing position. 1 = Perineal dermatome innervated by sacral nerves S3 and S4. 2 = Tail dermatome innervated by sacral nerves originating from the coccyx to sacral nerve S3. 3 = Dorsal hind limb dermatome innervated by sacral nerves originating from the coccyx to sacral nerve S2.](image)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Lidocaine alone</th>
<th>Neostigmine (µg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Onset of analgesia (min)</td>
<td>5 ± 3</td>
<td>9 ± 2</td>
</tr>
<tr>
<td>Total duration of analgesia (min)†</td>
<td>70 ± 12</td>
<td>120 ± 15†</td>
</tr>
<tr>
<td>Onset of motor blockade (min)§</td>
<td>5 ± 4</td>
<td>10 ± 3</td>
</tr>
<tr>
<td>End of motor blockade (min)</td>
<td>65 ± 6</td>
<td>110 ± 8†</td>
</tr>
</tbody>
</table>

*Treatments were 2% lidocaine (0.2 mg/kg) without epinephrine and that dose of lidocaine coadministered with neostigmine methylsulfate at 3 doses (0.5, 1, and 2 µg/kg). †Total duration of analgesia included pinprick and thermal stimulations. §Within a row, value differs significantly ($P < 0.05$) from the value for the lidocaine alone treatment. $^*$Onset of motor blockade was defined as a score of 2 or 3.
lidocaine alone, all horses had similar time responses to both analgesia tests. However, with the neostigmine treatments, the horses had differences in the time responses for the painful stimuli (needle pinprick or thermal stimulation). For these treatments, the horses had a response (score ≥ 3) to a pinprick for 110 minutes; from 110 minutes until the end of the experiments (approx. 150 minutes), horses had no response (score 1 or 2) to the pinprick stimulus and thus responded only to the thermal stimulation.

Compared with baseline values, heart rate and arterial pressures (SAP, DAP, and MAP) did not change significantly after treatment with lidocaine alone or lidocaine coadministered with neostigmine at doses of 0.5 or 1 μg/kg (Table 2). Significant decreases in heart rate at 15 minutes, in DAP at 45 minutes, and in MAP at 30 to 120 minutes were observed with the 2 μg/kg dose of neostigmine. None of the treatments resulted in significant alterations in respiratory rate or rectal temperature.

Discussion

Epidural coadministration of neostigmine at doses of 0.5, 1, and 2 μg/kg with lidocaine (0.2 mg/kg) to horses induced an analgesic effect, but the effect was not dose dependent. Several studies have involved the use of neostigmine indicating the relevance of activation of cholinergic receptors in the spinal cord and induction of analgesia in humans and other animals. Because of a high incidence of adverse effects (e.g., nausea and vomiting) after intrathecal administration of analgesic doses of neostigmine, this route of administration is no longer used in humans. Nevertheless, studies have clearly revealed that these effects were not observed after epidural administration of neostigmine. To our knowledge, the study reported here was the first in which investigators evaluated the potential benefits of caudal epidural administration of a cholinesterase inhibitor for analgesia in horses, and we did not observe harmful effects.

Studies on epidural administration of neostigmine have yielded conflicting results regarding its efficacy. Investigators in a few studies found that the duration of postoperative analgesia did not differ between bupivacaine and bupivacaine-neostigmine treatments in children or between morphine and morphine-neostigmine treatments in dogs. Another study revealed that the interval to first administration of rescue analgesia in children was longer with neostigmine and bupivacaine, compared with that for bupivacaine alone. Results of the present study confirmed that the 3 doses of neostigmine (0.5, 1, and 2 μg/kg) combined with lidocaine and administered epidurally improved regional perineal analgesia in horses; however, the result was not dose dependent. Lidocaine is an anesthetic that is tolerated well by horses after epidural administration, but the short duration of analgesia is a limitation of a single epidural injection, particularly in the postoperative period. Neostigmine administered intraoperatively presumably acts by mimicking the release of acetylcholine from intrinsic cholinergic neurons located deep in the dorsal horn of the spinal cord, from which a dense network of fibers extends to the superficial dorsal horn.
Spinal muscarinic receptors (M1 and M2) are believed to be involved in the analgesic properties of neostigmine in the spinal cord.\textsuperscript{12,13} and α\textsubscript{2}-adrenergic receptor agonists in the spinal cord are also mediated in part by cholinergic activation.\textsuperscript{20}

Neostigmine is a hydrophilic substance, and approximately one-tenth of a dose administered epidurally penetrates into the CSF and spinal cord to provide effective analgesia. Therefore, 10 μg of intrathecally administered neostigmine would be equivalent to approximately a 100-μg dose of neostigmine administered epidurally (ie, 2 μg/kg for a 50-kg patient).\textsuperscript{13} Because there is no preestablished dose for epidural administration of neostigmine in horses, we used a low (0.5 μg/kg), intermediate (1 μg/kg), and high (2 μg/kg) dose in combination with lidocaine on the basis of results of studies in humans. Analysis of the results in the horses of the present study suggested that the 3 doses of neostigmine combined with lidocaine improved analgesia and increased the duration of analgesia, compared with results for lidocaine alone. It appears that there was peak potentiation of the duration of lidocaine's analgesic effect (2.5 hours) at a neostigmine dose of 1 μg/kg; increasing the dose to 2 μg/kg did not further prolong the duration of analgesia. The 0.5 μg/kg dose of neostigmine provided analgesia for 2 hours. When neostigmine alone was administered epidurally to humans, 1 μg/kg resulted in analgesia for approximately 6 hours, whereas the same dose coadministered with lidocaine resulted in analgesia for 8 hours after surgery.\textsuperscript{15} Investigators in another study\textsuperscript{29} found that epidural administration of neostigmine alone reduced the need for supplemental analgesia in dogs after ovariohysterectomy; however, the addition of neostigmine to morphine in that study did not potentiate the analgesic effect of the opioid. In studies\textsuperscript{14,19} conducted to evaluate epidurally administered neostigmine, the analgesic effect was evaluated during surgical manipulations (ie, hysterectomy) and during parturition when the painful stimulus had already stopped. This represents a clinical situation extremely different from the situation in the present study in which horses were subjected to standard painful stimuli until the end of the experiments. It is likely that the epidurally administered dose of lidocaine in the present study potentiated the analgesic effect of neostigmine in the horses. Surprisingly, these analgesic effects were not increased after administration of a higher dose of neostigmine coadministered with lidocaine.

Results of a study\textsuperscript{15} in humans suggest that a neostigmine dose of <1 μg/kg administered via the epidural route may be inadvisable because it would be ineffective. We chose to administer small doses of neostigmine in the present study because a high total volume of drugs injected into the epidural space in horses can cause severe adverse effects, such as ataxia and gastrointestinal alterations.\textsuperscript{20} A certain degree of motor weakness can result from intrathecal administration of cholinesterase inhibitors; this motor weakness is related to an acetylcholine effect on motor neurons that can potentiate the axonal conduction block caused by local anesthetics.\textsuperscript{14} In the present study, all treatments yielded moderate motor block in the horses, and the effects were more prolonged in treatments that contained neostigmine. Similar results were obtained in other studies\textsuperscript{19,20} in humans with different doses of epidurally administered neostigmine. It is possible that epidurally administered neostigmine enhances the motor blockade of lidocaine. In perioperative conditions, for which muscle relaxation can be beneficial for perineal surgery, motor weakness during analgesia after caudal epidural administration may be potentially harmful in horses.

Systemically, IV injection of a small dose of acetylcholine causes a decrease in blood pressure as a result of generalized vasodilatation, which usually is accompanied by reflex tachycardia.\textsuperscript{23} However, IV infusion of high doses of acetylcholine results in hypotension and bradycardia. Accumulation of acetylcholine following administration of an anticholinesterase drug is not specific to neuromuscular junctions. The muscarinic cholinergic effects are caused by inhibition of acetylcholinesterase at the sinus node, smooth muscle, and glands, which leads to bradycardia and other adverse effects such as hyperperistalsis and salivation.\textsuperscript{28} Acetylcholine causes dilatation of essentially all vascular beds, including the pulmonary and coronary vascular beds.\textsuperscript{27} After epidural administration of neostigmine in the present study, we observed that the highest dose induced a decrease in heart rate, DAP, and MAP, but only MAP was affected long term. These effects may have been attributable to the slow systemic absorption of neostigmine from the epidural space associated with the sympathetic block induced by the lidocaine. In a study\textsuperscript{29} of epidurally administered neostigmine (75 to 300 μg) in women, investigators detected a high incidence of hypotension but not bradycardia.

One important adverse effect of systemic and intrathecal administration of neostigmine is increased intestinal motility, which in turn may lead to diarrhea, nausea, and vomiting.\textsuperscript{14,18,30} Surprisingly, these adverse gastrointestinal effects are not detected after epidural administration of neostigmine.\textsuperscript{3,13,19} As in those previous studies, we did not observe adverse gastrointestinal effects after epidural administration of neostigmine in horses of the present study. Regarding these contradictory findings, it is believed that cholinergic effects from epidural administration of neostigmine is mainly related to a spinal mechanism, although some supraspinal effects cannot be excluded.\textsuperscript{3}

Neostigmine, with an intermediate dose of 1 μg/kg, coadministered in the caudal epidural space with lidocaine (0.2 mg/kg) resulted in an analgesic effect (duration, 2.5 hours) with minimal adverse effects in standing conscious geldings. Additional studies are needed to determine whether neostigmine has a role as an adjuvant for postoperative pain management when coadministered with other anesthetic or analgesic drugs via the caudal epidural space in geldings.


b. Acepran, Univet SA, Indústria Veterinária, São Paulo, Brazil.

c. Perican, B Braun, São Gonçalo, Brazil.

d. Portex epidural catheter, Smiths Medical ASD Inc, Keene, NH.

e. Super Bonder, Henke, Itapevi, Brazil.


Appendix appears on the next page
Appendix
Scoring system used to assess analgesia, behavior, and motor blockade in horses.

<table>
<thead>
<tr>
<th>Score</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Analgesia</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Normal response; kicking or similar strong reaction to painful stimulus</td>
</tr>
<tr>
<td>2</td>
<td>Mild analgesia; no immediate response to skin pinprick, but tail swishing, low whole-body reaction, and turning toward site of painful stimulus</td>
</tr>
<tr>
<td>3</td>
<td>Moderate analgesia; no tail swishing, no response to skin and deep muscle pinprick, and no whole-body reaction but restlessness</td>
</tr>
<tr>
<td>4</td>
<td>Complete analgesia; calm and indifferent to painful stimulus</td>
</tr>
<tr>
<td>Behavior</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Calm and alert, occasional head movements, and not reluctant to move</td>
</tr>
<tr>
<td>2</td>
<td>Restless with a slight decrease in head height with drooping of the upper eyelids</td>
</tr>
<tr>
<td>3</td>
<td>Excitation, continuous body movements, and abnormal facial expression</td>
</tr>
<tr>
<td>Motor blockade</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>Standing quietly with no change in limb position and no other signs</td>
</tr>
<tr>
<td>2</td>
<td>Not reluctant to move, no tail movements, loss of anal reflex, and vulvar and vaginal relaxation in females</td>
</tr>
<tr>
<td>3</td>
<td>Presence or absence of ataxia (on the basis of a visual analogue scale) with the horse restrained in a stocks and then by walking after treatment</td>
</tr>
<tr>
<td>4</td>
<td>Recumbent</td>
</tr>
</tbody>
</table>