Effects of perineural administration of ropivacaine combined with perineural or intravenous administration of dexmedetomidine for sciatic and saphenous nerve blocks in dogs

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COMBINING a local anesthetic with dexmedetomidine, a potent α₂-adrenoceptor agonist, has gained popularity for locoregional anesthesia in human medicine. Such combinations have been shown to prolong sensory nerve blockade, enhance patients’ satisfaction, and reduce pain and postoperative morbidity requirements, and experimental studies with high doses of dexmedetomidine have shown that it does not induce axon or myelin degeneration and confirmed that it is safe to use when combined with local anesthetics. In addition, several clinical trials involving human patients have supported the use of long-acting, amide-type local anesthetics in combination with dexmedetomidine.

The optimal dose of dexmedetomidine needed to prolong locoregional anesthesia is unclear, and previous studies in people have used dexmedetomidine doses of 100 µg/site, 0.75 µg/kg, and 2 µg/kg. The minimum dose of dexmedetomidine needed to efficaciously prolong sensory nerve blockade remains to be determined. Further, whether the effects of dexmedetomidine are due to local or systemic actions of the drug remains unknown. In humans, IV administration of dexmedetomidine prolongs the effects of nerve blocks in a manner similar to that seen when the drug is administered peripherally. However, dexmedetomidine can induce effects such as bradycardia and sedation, and these effects may be

OBJECTIVE
To evaluate the effects of using ropivacaine combined with dexmedetomidine for sciatic and saphenous nerve blocks in dogs.

ANIMALS
7 healthy adult Beagles.

PROCEDURES
In phase 1, dogs received each of the following 3 treatments in random order: perineural sciatic and saphenous nerve injections of 0.5% ropivacaine (0.4 mL/kg) mixed with saline (0.9% NaCl) solution (0.04 mL/kg; DEX0PN), 0.5% ropivacaine mixed with dexmedetomidine (1 µg/kg; DEX1PN), and 0.5% ropivacaine mixed with dexmedetomidine (2 µg/kg; DEX2PN). In phase 2, dogs received perineural sciatic and saphenous nerve injections of 0.5% ropivacaine and an IV injection of diluted dexmedetomidine (1 µg/kg; DEX1IV). For perineural injections, the dose was divided equally between the 2 sites. Duration of sensory blockade was evaluated, and plasma dexmedetomidine concentrations were measured.

RESULTS
Duration of sensory blockade was significantly longer with DEX1PN and DEX2PN, compared with DEX0PN; DEX1IV did not prolong duration of sensory blockade, compared with DEX0PN. Peak plasma dexmedetomidine concentrations were reached after 15 minutes with DEX1PN (mean ± SD, 348 ± 200 pg/mL) and after 30 minutes DEX2PN (816 ± 607 pg/mL), and bioavailability was 54 ± 40% and 73 ± 43%, respectively. The highest plasma dexmedetomidine concentration was measured with DEX1IV (1,032 ± 415 pg/mL) 5 minutes after injection.

CONCLUSIONS AND CLINICAL RELEVANCE
Results suggested that perineural injection of 0.5% ropivacaine in combination with dexmedetomidine (1 µg/kg) for locoregional anesthesia in dogs seemed to balance the benefit of prolonging sensory nerve blockade while minimizing adverse effects. (Am J Vet Res 2021;82:449–458)

ABBREVIATIONS

AUC Area under the curve
DEX0PN Perineural injection of 0.5% ropivacaine (0.4 mL/kg) mixed with saline (0.9% NaCl) solution
DEX1PN Perineural injection of 0.5% ropivacaine and IV injection of dexmedetomidine (1 µg/kg)
DEX2PN Perineural injection of 0.5% ropivacaine mixed with dexmedetomidine (2 µg/kg)
more prominent following IV rather than perineural administration.

In dogs, studies involving perineural administration of dexmedetomidine for locoregional anesthesia are rare. Ultrasound-guided sciatic and saphenous nerve blocks are performed regularly in dogs, but the local anesthetics used for these blocks are not commonly mixed with dexmedetomidine. Thus, plasma concentrations following perineural administration of dexmedetomidine and the optimal dose of dexmedetomidine for perineural injection to significantly prolong nerve blocks in dogs are unknown.

The purpose of the study reported here was to evaluate the effects of using ropivacaine combined with dexmedetomidine for sciatic and saphenous nerve blocks in dogs. The primary objective was to compare nerve block durations after perineural injection of ropivacaine alone, perineural injections of ropivacaine and dexmedetomidine, and perineural injection of ropivacaine combined with IV administration of dexmedetomidine. The secondary objective was to determine plasma concentrations of dexmedetomidine following perineural injection in combination with ropivacaine. We hypothesized that the combination of ropivacaine and dexmedetomidine would significantly prolong nerve blockade duration, compared with ropivacaine alone, and that this effect would not be associated with the plasma concentration of dexmedetomidine.

**Materials and Methods**

**Sample size calculation**

The study protocol was approved by the commission for the ethical use of animals at the University of Liège Faculty of Veterinary Medicine (No. 16-1887). A sample size calculation for a continuous outcome and superiority trial was performed with online software. Power and $\alpha$ were set at 80% and 5%, respectively. The mean ± SD duration of sensory ulnar nerve blockade (9.1 ± 3.3 hours) induced in people by perineural injection of 22.5 mg of 0.5% ropivacaine combined with 100 µg of dexmedetomidine versus the mean duration of sensory nerve blockade (4 hours) induced by perineural injection of 0.5% ropivacaine alone (0.2 mL/kg) was used for the calculation. The sample size calculation revealed that a total of 7 dogs were required for each treatment.

**Animals**

Seven sexually intact adult Beagles (4 males and 3 females) with a mean ± SD weight of 16.9 ± 2.3 kg were used in the study. All dogs were considered healthy on the basis of findings of a complete physical examination, including orthopedic and neurologic examinations, and were classified as American Society of Anesthesiologists physical status I or II. All dogs were 10 years old except for 1 female, which was 6 years old. The dogs were housed in a kennel, fed dry commercial food once daily, and provided water ad libitum.

On study days, dogs were moved to a separate room located in the same building as the kennel, where they were kept in individual cages for nerve block evaluations. Dogs were fed 2 hours after recovery from anesthesia and were returned to the kennel after the last evaluation.

The study consisted of 2 phases. In the first phase, dogs underwent 3 experimental treatments with a minimum washout period of 1 week between treatments. The second phase was performed 8 months after the first phase and consisted of a single experimental treatment.

**Anesthesia**

On study days, a 22-gauge catheter was inserted in a cephalic vein, and lactated Ringer solution was administered at a rate of 5 mL/kg/h. Anesthesia was induced with propofol (4 to 8 mg/kg, IV), and an appropriately sized, cuffed endotracheal tube was placed in the trachea. Dogs were connected to a circle breathing system, and anesthesia was maintained with sevoflurane (2% to 3%) in oxygen. Monitoring was performed with a multiparameter monitor and included pulse oximetry, noninvasive blood pressure measurement with a size 3 pediatric cuff placed around a front limb, 3-lead base-apex electrocardiography, and measurement of end-tidal CO$_2$ and sevoflurane concentrations. A 19-gauge jugular vein catheter was placed to enable stress-free blood sample collection following anesthetic recovery.

**Sciatic and saphenous nerve blocks**

A 500-µg/mL dexmedetomidine solution was diluted with saline (0.9% NaCl) solution in a 1:10 ratio to obtain a 50-µg/mL solution. In phase 1 of the study, an online randomization generator was used to assign dogs to receive 3 treatments in random order. Treatments consisted of perineural sciatic and saphenous nerve injections of 0.5% ropivacaine (0.4 mL/kg) mixed with saline solution (0.04 mL/kg; DEX0PN treatment), 0.5% ropivacaine (0.4 mL/kg) mixed with diluted dexmedetomidine (1 µg/kg [0.02 mL/kg of the 50-µg/mL dexmedetomidine solution]) and saline solution (0.02 mL/kg; DEX1PN treatment), and 0.5% ropivacaine (0.4 mL/kg) mixed with diluted dexmedetomidine (2 µg/kg [0.04 mL/kg of the 50-µg/mL dexmedetomidine solution]; DEX2PN treatment). In phase 2 of the study, all dogs received perineural sciatic and saphenous nerve injections of 0.5% ropivacaine (0.4 mL/kg) and an IV injection of diluted dexmedetomidine (1 µg/kg [0.02 mL/kg of the 50-µg/mL dexmedetomidine solution]; DEX1IV treatment). The perineural sciatic and saphenous nerve injections were performed first, and dexmedetomidine was injected through the cephalic vein catheter immediately afterwards over 60 seconds, and dogs were allowed to recover from anesthesia. For all treatments, the total volume of drug administered perineurally was divided equally between the sciatic and saphenous nerve sites.
For the perineural injections, the areas over the saphenous and sciatic nerves were clipped and disinfected. Sterile contact gel was applied, and ultrasound-guided\textsuperscript{6} sciatic and saphenous nerve blocks were performed with an insulated needle\textsuperscript{6} by means of a standard approach.\textsuperscript{13} An electric nerve stimulator\textsuperscript{6} was used to confirm correct needle placement for the sciatic nerve block. The needle and injection line were flushed with 0.7 mL of saline solution after each perineural injection. A single experienced board-certified veterinary anesthetist (VM) performed all nerve blocks and was unaware of which treatment was administered on each study day for phase 1.

**Postanesthetic monitoring**

Once all injections were completed, sevoflurane administration was discontinued, and the dogs were allowed to recover from anesthesia. Time from discontinuation of sevoflurane administration to extubation was recorded. Heart rate was assessed every 15 minutes by means of auscultation of the left thoracic wall until it had returned to the preanesthetic baseline rate. A sedation score (14 = maximum sedation, 0 = no sedation, and negative values = awake)\textsuperscript{14} was assigned every 15 minutes until a score of 0 or a negative score was assigned.

Sciatic and saphenous nerve blocks were assessed by means of nociception, locomotion, and proprioception tests every 15 minutes after perineural injections until recovery from nerve blockade was documented. Testing was performed by an investigator (VM) who was blinded to the particular treatment performed during phase 1 of the study. Nociception was assessed by clamping the skin with a needle holder for 2 seconds over the caudal part of the thigh region (to evaluate the sciatic nerve), over the dorsal part of the fourth metatarsal region (to evaluate the fibular nerve), over the plantar part of the fourth metatarsal region (to evaluate the tibial nerve), and over the medial part of the distal aspect of the femur (to evaluate the saphenous nerve). Behaviors such as barking, crying, immediate withdrawal of the limb, attempting to escape, and actively looking at the stimulated site were assigned a score of 1 (sensory feeling present); mild reactions such as slow withdrawal of the limb or slow head movement toward the stimulated area were assigned a score of 2 (sensory feeling partially present); and an absence of any reaction was assigned a score of 3 (sensory feeling absent).

Locomotion was scored on a scale from 1 to 3, with 1 representing a normal gait, 2 representing an abnormal gait with some missteps observed, and 3 representing an abnormal gait with dragging of the limb.

Proprioception was assessed on the basis of spontaneous repositioning of the limb after the dorsal part of the metatarsal region was positioned on the ground. Proprioception was scored on a scale from 1 to 3, with 1 representing immediate repositioning, 2 representing reduced or retarded repositioning, and 3 representing no repositioning.

Nociception, locomotion, and proprioception tests were performed every 15 minutes until at least 2 successive scores of 1 were recorded for each test. Times elapsed from the perineural injection until the first score of 2 or 3 (blockade) and the first score of 3 (complete blockade) were recorded as onset times. Times with scores of 2 or 3 and with a score of 3 only were defined as the durations of sensory blockade and complete sensory blockade, respectively.

**Pharmacokinetic analysis**

To assess plasma dexmedetomidine concentrations, 10 mL of blood was withdrawn from the jugular catheter, 2 mL of blood was collected for analysis, and the initial 10-mL blood sample was returned to the dog to avoid excessive blood loss. The jugular catheter was then flushed with 2 mL of saline solution. Blood samples were collected 15, 30, 60, 90, 120, 180, 240, 360, and 480 minutes after injections were completed (an additional blood sample was collected 5 minutes after injections were completed following the DEX\textsuperscript{1IV} treatment). Samples were placed in heparinized tubes and immediately centrifuged (4 minutes at 2,200 X g). Plasma samples were collected with a pipette and frozen at -80°C until transport on dry ice to the Medical University of Gdańsk.

Plasma samples were analyzed by means of reverse-phase high-performance liquid chromatography coupled with triple-quadrupole mass spectrometry detection and a validated method developed for analysis of dexmedetomidine in pediatric patients after IV administration of the drug.\textsuperscript{15} Because of possible species differences, the most crucial analytic method parameters (specificity, linearity, intra- and interday precision, and accuracy) were revalidated. Calibration curves for dexmedetomidine concentration were made by spiking canine plasma with dexmedetomidine to obtain 7 dexmedetomidine concentrations (5, 10, 50, 100, 500, 1,000, and 2,500 pg/mL). Precision and accuracy of the method were assessed with 3 quality control plasma samples at 3 concentrations (20, 200, and 2,000 pg/mL). Specificity of the method was assessed by analyzing control canine plasma and canine plasma spiked with dexmedetomidine for dexmedetomidine ion transition.

Plasma concentrations of dexmedetomidine were plotted against time, and standard formulas were used on the basis of the best compartmental model that fit the data to calculate bioavailability ([\text{AUC}_{\text{IV}}/\text{AUC}_{\text{PN}}]) \times [\text{DOSIE}_{\text{IV}}/\text{DOSIE}_{\text{PN}}] \times 100, where \text{AUC}_{\text{IV}} and \text{AUC}_{\text{PN}} represent the area under the dexmedetomidine concentration-vs-time curve following perineural and IV injection, respectively), elimination half-life, plasma clearance, and volume of distribution of dexmedetomidine.

**Statistical analysis**

Data distributions were assessed for normality with the Kolmogorov-Smirnov test. Body weight, anesthesia time (ie, time from anesthetic induction with
propofol to discontinuation of sevoflurane administration), time to extubation, heart rate, onset and duration of nerve blockade, proprioception and locomotion scores, plasma dexmedetomidine concentration, bioavailability, and half-life were compared between treatments by means of 2-way repeated-measures ANOVA and post hoc Tukey tests. Sedation scores were assessed within a group with the Wilcoxon signed rank test. All statistical analyses were performed with standard software. Values of $P < 0.05$ were considered significant.

**Results**

One dog was euthanized after a diagnosis of hepatic tumor metastasis was made. Data for this dog were obtained only for the DEX0PN and DEX1PN treatments.

No significant differences in body weight (mean ± SD: DEX0PN, 16.9 ± 2.3 kg; DEX1PN, 16.9 ± 2.3 kg; DEX2PN, 16.6 ± 2.4 kg; DEX1IV, 14.5 ± 1.6 kg; $P = 0.205$) or anesthesia time (DEX0PN, 26.1 ± 7.8 minutes; DEX1PN, 21.4 ± 6.1 minutes; DEX2PN, 28.2 ± 9.1 minutes; DEX1IV, 25.2 ± 8.6 minutes; $P = 0.484$) were observed among treatments.

Extubation time was significantly longer after the DEX1IV treatment ($13.0 ± 4.2$ minutes) than after the DEX1PN ($5.1 ± 2.3$ minutes; $P = 0.001$) and DEX0PN ($5.7 ± 2.3$ minutes; $P = 0.002$) treatments. Extubation time was also significantly ($P = 0.014$) longer after the DEX2PN treatment ($10.5 ± 4.2$ minutes) than after the DEX1PN treatment.

**Analytic method validation**

Validation of the method for measuring plasma dexmedetomidine concentration revealed that the method was linear over a range from 5 to 2,500 pg/mL, with a correlation coefficient $> 0.999$, and had acceptable specificity (Figure 1). Intra- and interday coefficients of variation for precision were < 10% (Table 1). With regard to accuracy, differences between measured and nominal concentrations were < 10%. On the basis of validation data, the method was concluded to be selective, linear over the tested concentration range, precise, and accurate; the validation values fell within the bioanalytical methods criteria proposed by the US FDA.

**Pharmacokinetic analyses**

Plasma samples obtained from 1 dog after the DEX1IV treatment were excluded from analyses because measured dexmedetomidine concentrations were low or undetectable. These results were not considered to be reliable, and sufficient plasma to repeat the analyses was not available. Potential causes of the unreliable results included blood sampling errors, perivascular injection, an error in dexmedetomidine dose calculation, and analysis errors.

Examination of the plasma dexmedetomidine concentration-versus-time curves (Figure 2) suggested that a 2-compartment model best fit the data (Table 2). For the DEX1IV treatment, mean ± SD plasma dexmedetomidine concentration 5 minutes after injection was $1,032 ± 415$ pg/mL. Plasma concentrations peaked 15 minutes after injection with the DEX1PN treatment ($348 ± 200$ pg/mL) and 30 minutes after injection with the DEX2PN treatment ($819 ± 607$ pg/mL). Plasma dexmedetomidine concentrations were significantly lower with the DEX1IV treatment than with the DEX2PN treatment 120 ($P = 0.021$), 180 ($P = 0.005$), 240 ($P = 0.008$), 360 ($P = 0.006$), and 480 ($P = 0.048$) minutes after injection and were signifi-
Sedation scores

Sedation scores were significantly different from the baseline score (score, 0) 15 minutes after injection with the DEX1PN (mean, 1; range, 0 to 7; \( P = 0.034 \)), DEX2PN (mean, 3; range, 0 to 14; \( P = 0.006 \)), and DEX1IV (mean, 13; range, 6 to 14; \( P = 0.034 \)) treatments and 30 minutes after injection with the DEX2PN (mean, 2; range, 0 to 13; \( P = 0.006 \)) and DEX1IV (mean, 5; range, 2 to 9; \( P = 0.035 \)) treatments. Following the DEX0PN treatment, no significant changes in sedation scores from baseline scores were identified.

With the DEX1IV treatment, sedation scores were significantly higher 15 minutes after injection than were scores obtained following the DEX0PN (mean, 0; range, 0 to 2; \( P = 0.003 \)), DEX1PN (mean, 0.005), and DEX2PN (mean, 0.050) treatments and were significantly higher 30 minutes after injection than were scores obtained following the DEX0PN (mean, 0; range, 0 to 2; \( P = 0.003 \)) and DEX1PN (mean, 0; range, 0 to 5; \( P = 0.010 \)) treatments. With the DEX2PN treatment, sedation scores were significantly higher 15 minutes after injection than were scores obtained following the DEX0PN treatment (\( P = 0.043 \)) and were significantly higher 30 minutes after injection than were scores obtained following the DEX1PN treatment (\( P = 0.048 \)). No significant differences were observed in sedation scores between the DEX0PN and DEX1PN treatments at any time.

Heart rate

Mean ± SD heart rates 15 minutes after discontinuation of sevoflurane administration were 101 ± 9 beats/min with the DEX1PN treatment, 83 ± 24 beats/min with the DEX2PN treatment, 107 ± 35 beats/min with the DEX0PN treatment, and 74 ± 15 beats/min with the DEX1IV treatment. A significant difference was detected between baseline heart rate and heart rate 15 minutes after discontinuation of anesthesia with the DEX1IV treatment (\( P = 0.002 \)) and between the DEX0PN and DEX1IV treatments 15 minutes after discontinuation of anesthesia (\( P = 0.025 \)).

Table 1—Results of validation of a method (reverse-phase high-performance liquid chromatography coupled with triple-quadrupole mass spectrometry detection) for measuring dexmedetomidine concentration in plasma samples from dogs.

<table>
<thead>
<tr>
<th>Nominal concentration (pg/mL)</th>
<th>Measured concentration (pg/mL)</th>
<th>SD (pg/mL)</th>
<th>Precision (CV)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraday evaluation (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>21.6</td>
<td>1.8</td>
<td>8.54</td>
<td>107.82</td>
</tr>
<tr>
<td>200</td>
<td>219.4</td>
<td>11.1</td>
<td>5.07</td>
<td>109.69</td>
</tr>
<tr>
<td>2,000</td>
<td>2,077.8</td>
<td>57.9</td>
<td>2.79</td>
<td>103.89</td>
</tr>
<tr>
<td>Interday evaluation (n = 20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>20.5</td>
<td>1.8</td>
<td>9.01</td>
<td>102.51</td>
</tr>
<tr>
<td>200</td>
<td>215.4</td>
<td>14.0</td>
<td>6.49</td>
<td>107.69</td>
</tr>
<tr>
<td>2,000</td>
<td>2,110.2</td>
<td>118.1</td>
<td>5.59</td>
<td>105.51</td>
</tr>
</tbody>
</table>

CV = Coefficient of variation.

Nociception

No significant differences in onset times for tibial (\( P = 0.132 \)), fibular (\( P = 0.385 \)), saphenous (\( P = 0.130 \)), and sciatic (\( P = 0.060 \)) nerve sensory blockade (ie, time from the perineural injection until the first nociception score of 2 or 3) were observed among treatments (Table 3). The onset time for complete tibial (\( P = 0.360 \)), fibular (\( P = 0.274 \)), and saphenous (\( P = 0.181 \)) nerve sensory blockade (ie, time from the perineural injection until the first nociception score of 3) was also not significantly different among treatments. The only significant difference in onset times was that the onset time for complete sciatic nerve sensory blockade was significantly (\( P = 0.007 \)) shorter with the DEX2PN treatment than with the DEX0PN treatment.

Durations of sensory blockade (ie, time during which nociception scores of 2 or 3 were recorded)
and complete sensory blockade (ie, time during which only nociception scores of 3 were recorded) for the tibial nerve were significantly longer with the DEX1PN treatment than with the DEX0PN (P = 0.003 and P = 0.003, respectively) or the DEX1IV (P = 0.012 and P = 0.006, respectively) treatments. Durations of fibular nerve sensory blockade were significantly longer with the DEX1PN and DEX2PN treatments than with the DEX0PN (P = 0.010 and P = 0.010, respectively) or DEX1IV (P = 0.015 and P = 0.013, respectively) treatments. Durations of complete fibular nerve sensory blockade were significantly longer with the DEX1PN (P = 0.011) and DEX2PN (P = 0.006) treatments than with the DEX0PN treatment.

### Table 2—Pharmacokinetic variables for plasma dexmedetomidine concentration in dogs that underwent sciatic and saphenous nerve blocks with perineural injection of ropivacaine in combination with perineural or IV injection of dexmedetomidine.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DEX1PN (n = 7)</th>
<th>DEX2PN (n = 6)</th>
<th>DEX1IV (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioavailability (%)</td>
<td>54 ± 40</td>
<td>73 ± 43</td>
<td>NA</td>
</tr>
<tr>
<td>Half-life (min)</td>
<td>147 ± 98</td>
<td>258 ± 119</td>
<td>93 ± 50</td>
</tr>
<tr>
<td>Plasma clearance (mL/kg/min)</td>
<td>NA</td>
<td>NA</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>Volume of distribution (L/kg)</td>
<td>NA</td>
<td>NA</td>
<td>0.6 ± 0.4</td>
</tr>
</tbody>
</table>

Dogs received perineural sciatic and saphenous nerve injections of 0.5% ropivacaine (0.4 mL/kg) combined with perineural injection of a lower (1 µg/kg; DEX1PN) or higher (2 µg/kg; DEX2PN) dose of dexmedetomidine or with IV administration of dexmedetomidine (1 µg/kg; DEX1IV). For perineural injections, the dose was divided equally between the 2 sites. Blood samples were collected up to 480 minutes after injections were completed for measurement of plasma dexmedetomidine concentrations. Data are given as mean ± SD.

NA = Not applicable.

### Table 3—Median (range; minutes) onset times and durations of sensory blockade and complete sensory blockade of the tibial, fibular, saphenous, and sciatic nerves in dogs that underwent sciatic and saphenous nerve blocks with perineural injection of ropivacaine alone (DEX0PN) or ropivacaine in combination with perineural or IV injection of dexmedetomidine.

<table>
<thead>
<tr>
<th>Variable</th>
<th>DEX0PN</th>
<th>DEX1PN</th>
<th>DEX2PN</th>
<th>DEX1IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibial nerve Blockade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>30 (15–60)</td>
<td>30 (15–45)</td>
<td>15 (15–60)</td>
<td>15 (15–45)</td>
</tr>
<tr>
<td>Duration</td>
<td>190 (105–235)</td>
<td>400 (160–655)††</td>
<td>332 (190–520)</td>
<td>205 (135–310)</td>
</tr>
<tr>
<td>Complete blockade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>45 (15–105)</td>
<td>45 (15–60)</td>
<td>30 (15–60)</td>
<td>52 (15–105)</td>
</tr>
<tr>
<td>Duration</td>
<td>75 (45–175)</td>
<td>340 (15–490)††</td>
<td>190 (115–325)</td>
<td>102 (15–280)</td>
</tr>
<tr>
<td>Fibular nerve Blockade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>30 (15–60)</td>
<td>15 (15–45)</td>
<td>15 (15–30)</td>
<td>15 (15–45)</td>
</tr>
<tr>
<td>Duration</td>
<td>235 (145–325)</td>
<td>415 (160–685)††</td>
<td>347 (235–550)††</td>
<td>197 (175–310)</td>
</tr>
<tr>
<td>Complete blockade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>45 (15–105)</td>
<td>30 (15–45)</td>
<td>22 (15–60)</td>
<td>52 (15–60)</td>
</tr>
<tr>
<td>Duration</td>
<td>130 (60–190)</td>
<td>355 (90–505)†</td>
<td>227 (130–400)†</td>
<td>137 (60–295)</td>
</tr>
<tr>
<td>Saphenous nerve Blockade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration</td>
<td>250 (75–430)</td>
<td>445 (370–490)††</td>
<td>400 (160–595)†</td>
<td>280 (160–310)</td>
</tr>
<tr>
<td>Complete blockade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>45 (15–90)</td>
<td>30 (15–150)</td>
<td>15 (15–30)</td>
<td>45 (15–75)</td>
</tr>
<tr>
<td>Duration</td>
<td>190 (15–340)</td>
<td>310 (230–415)††</td>
<td>332 (135–550)††</td>
<td>197 (75–265)</td>
</tr>
<tr>
<td>Sciatic nerve Blockade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>30 (15–75)</td>
<td>15 (15–45)</td>
<td>15 (15–30)</td>
<td>15 (15–45)</td>
</tr>
<tr>
<td>Duration</td>
<td>145 (105–310)</td>
<td>340 (160–520)†</td>
<td>340 (190–505)†</td>
<td>205 (105–310)</td>
</tr>
<tr>
<td>Complete blockade</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Onset</td>
<td>60 (15–105)</td>
<td>60 (15–75)</td>
<td>15 (15–30)‡</td>
<td>15 (15–60)</td>
</tr>
<tr>
<td>Duration</td>
<td>75 (15–135)</td>
<td>190 (75–445)†</td>
<td>310 (175–460)†</td>
<td>162 (75–280)</td>
</tr>
</tbody>
</table>

Nociception was assessed every 15 minutes by clamping the skin with a needle holder for 2 seconds over the caudal part of the thigh region (to evaluate the sciatic nerve), over the dorsal part of the fourth metatarsal region (to evaluate the fibular nerve), over the plantar part of the fourth metatarsal region (to evaluate the tibial nerve), and over the medial part of the distal aspect of the femur (to evaluate the saphenous nerve) and was scored as 1 (sensory feeling present), 2 (sensory feeling partially present), or 3 (sensory feeling absent). Onset time was defined as time from perineural injection to the first score of 2 or 3 (blockade) or the first score of 3 (complete blockade). Duration was defined as time during which scores of 2 or 3 (blockade) or only 3 (complete blockade) were recorded.

*Significantly (P < 0.05) different from the value for the DEX0PN treatment. †Significantly (P < 0.05) different from the value for the DEX1IV treatment.

See Table 2 for remainder of key.
Durations of saphenous nerve sensory blockade and complete sensory blockade were significantly longer with the DEX1PN treatment than with the DEX0PN (P = 0.002 and P = 0.015, respectively) and DEX1IV (P < 0.001 and P = 0.005, respectively) treatments. Duration of saphenous nerve sensory blockade was significantly longer with the DEX2PN treatment than with the DEX1PN (P = 0.037) treatment, and duration of complete sensory blockade was significantly longer with the DEX2PN treatment than with the DEX0PN (P = 0.054) and DEX1IV (P = 0.027) treatments.

Durations of sciatic nerve sensory blockade and complete sensory blockade were significantly longer with the DEX1PN (P = 0.015 and P = 0.013, respectively) and DEX2PN (P = 0.011 and P < 0.001, respectively) treatments than with the DEX0PN treatment.

**Proprioception and locomotion**

No significant differences in onset times and durations of proprioceptive and locomotor deficits were observed within or between groups (Table 4).

**Complications**

A vessel was punctured during administration of an ultrasound-guided saphenous nerve block in 1 dog. Blood was aspirated in the needle hub before the perineural injection was performed. The needle was immediately withdrawn and reoriented to avoid intravascular injection. Three dogs sustained injuries in the interdigital space during the second treatment of phase 1 of the study. The injury was not apparent on the day experiments were carried out; however, the paw was red, warm, and swollen the following day. The interdigital space was mildly ulcerated in 2 of the dogs and moderately ulcerated in 1. The injury was attributed to excessive weight bearing on the dorsal aspect of the digits (paw knuckling) and excessive dragging of the limb. Dogs were examined twice a day by a veterinarian, and appropriate care was provided. The hair was clipped, and the injury was disinfected with povidone-iodine solution. Dogs were treated with carprofen PO for 5 days and with amoxicillin–clavulanic acid PO for 10 days. The third experimental treatment was not started until after healing was complete. For all subsequent experiments, a soft protective bandage was applied over the digits and a soft mattress was placed in the cage for all dogs. The bandage was removed for each assessment and replaced immediately afterward. The paw was carefully examined visually and by palpation for injury at each evaluation time and 3 times a day for 2 days after experiments.

**Discussion**

Results of the study reported here indicated that, in dogs, performing sciatic and saphenous nerve blocks with a combination of 0.5% ropivacaine (0.4 mL/kg) and dexmedetomidine (1 or 2 µg/kg [0.5 or 1 µg/kg/nerve block site]) significantly prolonged sciatic, fibular, tibial, and saphenous nerve sensory blockade without increasing the duration of motor blockade or proprioception deficits, compared with performing the same blocks with 0.5% ropivacaine alone. A systematic review of adjuvants used to prolong peripheral nerve blocks in humans concluded that nerve blockade could be prolonged by 50 minutes to 4.5 hours when dexmedetomidine was combined with ropivacaine. Our findings were similar to results of previous studies involving humans, but doses and adverse effects of dexmedetomidine and concentrations of ropivacaine varied greatly among these studies. In a previous study, a dose of dexmedetomidine equaling 0.1 µg/kg/nerve block site in...
combination with bupivacaine did not significantly prolong sciatic and femoral nerve blockade in dogs. For this reason, we elected to evaluate higher doses of dexmedetomidine (1 and 2 µg/kg). Doses used in the present study were also chosen on the basis of current practice in human medicine.\textsuperscript{17} Importantly, in a study\textsuperscript{9} that compared doses of dexmedetomidine equaling 1, 1.5, and 2 µg/kg for interscalene brachial plexus blocks in people, the researchers suggested that 2 µg/kg might be the optimal dose but that it was associated with an increased risk of hypotension.

Perineural administration of dexmedetomidine at a dose of 1 µg/kg has the potential to induce sedation, bradycardia, and hypotension in humans.\textsuperscript{18,19} In the present study, heart rate did not significantly decrease when dexmedetomidine was administered perineurally but did decrease when dexmedetomidine was administered IV. This might suggest that adverse effects such as bradycardia might be more likely when dexmedetomidine is administered IV for locoregional anesthesia in dogs. Sedation scores were significantly higher with the DEX2PN and DEX1IV treatments up to 30 minutes after anesthesia was discontinued, and extubation time was significantly longer with the DEX1IV treatment. Moderate and deep sedation in human patients have been associated with plasma dexmedetomidine concentrations of 200 to 300 pg/mL and 1,900 pg/mL, respectively.\textsuperscript{20,21} In the present study, similar concentrations were measured after perineural and IV administration of dexmedetomidine 5, 15, 30, and even up to 60 minutes after injection. In people, plasma dexmedetomidine concentration after administration of a brachial plexus block with 150 µg of dexmedetomidine was 640 pg/mL 30 minutes after injection and progressively decreased by 2 pg/mL/min.\textsuperscript{6} This was similar to plasma concentrations measured following the DEX2PN treatment in the present study, suggesting that undesired systemic effects of dexmedetomidine could be avoided or reduced by using the DEX1PN treatment. The objective with locoregional anesthesia is to maintain a low plasma concentration of dexmedetomidine. A 1-µg dose (0.5 µg/kg/nerve block site) of dexmedetomidine/kg seemed to be effective for prolonging sensory nerve blockade while minimizing the likelihood of adverse systemic effects, and a 2-µg/kg dose did not provide significant advantages, compared with a 1-µg/kg dose. It is possible that a dose lower than 1 µg/kg but higher than 0.2 µg/kg, as evaluated in another study,\textsuperscript{15} might be effective to prolong sciatic and saphenous nerve sensory blockade. Bioavailability was lower with the DEX1PN treatment, and our findings suggested that perineural rather than the IV administration of dexmedetomidine for locoregional anesthesia would be better.

In the present study, IV administration of dexmedetomidine did not significantly prolong peripheral nerve blockade, compared with the control treatment (DEX0PN), which was in opposition to findings in humans.\textsuperscript{10} This difference might be explained by differences in study design, in that duration of analgesia and 24-hour cumulative morphine consumption were used as end points in the previous study. The differences in duration of sensory blockade following perineural and IV administration of dexmedetomidine might be linked to the mechanism of action by which dexmedetomidine prolongs locoregional anesthesia. Even though the exact mechanism remains unclear, a recent study has started to provide an answer. Andersen et al\textsuperscript{22} analyzed the effects of perineural dexmedetomidine administration in volunteers and observed that saphenous nerve blockade was prolonged when dexmedetomidine was combined with ropivacaine, compared with the duration with ropivacaine alone in the contralateral limb. They concluded that the mechanism of action of dexmedetomidine following perineural administration might be peripheral, which would be supported by our results. We observed that sensory nerve blockade was prolonged only when dexmedetomidine was injected perineurally and that effects were not associated with plasma concentrations of dexmedetomidine, which were initially lower and then similar to concentrations obtained following IV administration of dexmedetomidine. The local effects of α₂-adrenoceptor agonists, such as dexmedetomidine, following perineural administration are thought to be related to vasoconstriction, which can delay the absorption of ropivacaine and inhibit compound action potentials.\textsuperscript{23,24} Blockage of the hyperpolarization-activated cation current, thus keeping the nerve in a hyperpolarized state, is another potential explanation for the peripheral mechanism of action of dexmedetomidine.\textsuperscript{25} Dose-dependent central analgesia induced by dexmedetomidine seemed unlikely, as the duration of locoregional anesthesia was not significantly increased with the DEX1IV treatment. A centrally mediated antinociceptive effect of dexmedetomidine through stimulation of presynaptic α₂-adrenoceptors in the CNS seemed less probable but could not be excluded, and perineural injection of dexmedetomidine could be effective through the drug’s actions on the spinal cord.

Limitations of the present study included the small number of animals and the fact that some data were missing because 1 dog had to be euthanized, and measurement of plasma dexmedetomidine concentrations in 1 dog following the DEX1IV treatment could not be repeated. The puncture-related vessel damage and self-injuries may have influenced the behavioral results of the dogs. The fact that the investigator was not blinded during the DEX1IV treatment was another major limitation of the present study. After randomization, results regarding sensory blockade duration were evaluated with an unpaired t test. Significant differences were obtained for the DEX0PN versus the DEX1PN treatment (\(P = 0.001\)) and for the DEX0PN versus the DEX2PN treatment (\(P = 0.010\)) but not for the DEX1PN versus the DEX2PN treatment (\(P = 0.082\)). From this, we concluded that a dose equaling 1 µg of dexmedetomidine/kg was sufficient to
prolong sensory blockade duration; therefore, IV administration of this dose was performed to evaluate the effects of systemic administration (DEXIV treatment). The study was purposefully planned to optimize animal welfare. A Latin-square crossover study including 5 treatments, with an additional treatment involving IV administration of dexmedetomidine at a dose of 2 µg/kg, would have been ideal. However, this treatment was specifically not included to reduce the number of experiments per animal.

In conclusion, perineural injection of 0.5% ropivacaine in combination with dexmedetomidine (1 µg/kg) for locoregional anesthesia in dogs seemed to balance the benefit of prolonging sensory nerve blockade while minimizing adverse effects. At a dose of 1 µg/kg, the perineural route should be favored over the IV route to administer dexmedetomidine for locoregional analgesia. At the tested doses, the plasma concentrations of dexmedetomidine were low and only weakly associated with sensory blockade duration. These findings and the pharmacokinetic model might guide the route and dose of dexmedetomidine for locoregional analgesia to be assessed in clinical studies in dogs.

Acknowledgments
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The authors declare that there were no conflicts of interest.

Footnotes

b. S/5 Compact, Datex-Ohmeda, Helsinki, Finland.
c. Dexdomitor, Orion, Meชelen, Belgium.
e. Naropin, AstraZeneca, Dilbeek, Belgium.
f. Mindray, Schöneiche, Germany.
g. SonoPlex Stim cannula (21G, 10 cm), Pajunk, Geisingen, Germany.
h. TOF-watch, Organon, Dublin, Ireland.
i. RP-HPLC-QqQ/MS, Agilent Technology, Waldbronn, Germany.
j. Prism, version 5.03, GraphPad Software, San Diego, Calif.

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