Relationship between plasma dexmedetomidine concentration and sedation score and thermal threshold in cats

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Objective—To characterize the relationship between plasma dexmedetomidine concentration and the temperature difference between the thermal threshold and skin temperature (ΔT) and between plasma dexmedetomidine concentration and sedation score in healthy cats.

Animals—5 healthy adult spayed female cats.

Procedures—Cats received IV administrations of saline (0.9% NaCl) solution, dexmedetomidine (5, 20, or 50 µg/kg), or acepromazine (0.1 mg/kg). Blood samples were collected and thermal threshold and sedation score were determined before and at various times up to 8 hours after drug administration. In addition, cats received an IV infusion of dexmedetomidine that targeted a concentration achieving 99% of the maximum effect on ΔT.

Results—No change in ΔT over time was found for the saline solution and acepromazine treatments; ΔT increased for 45 minutes when cats received dexmedetomidine at 5 and 20 µg/kg and for 180 minutes when cats received dexmedetomidine at 50 µg/kg. No change in sedation score over time was found for saline solution. Sedation score increased for 120 minutes after cats received acepromazine and for 60, 120, and 180 minutes after cats received dexmedetomidine at 5, 20, and 50 µg/kg, respectively. The plasma dexmedetomidine concentration–effect relationships for the effect on ΔT and sedation score were almost identical. The plasma dexmedetomidine concentration after infusion was lower than targeted, and ΔT was not significantly affected.

Conclusions and Clinical Relevance—Dexmedetomidine administration to cats resulted in thermal analgesia and also profound sedation. These data may be useful for predicting the course of thermal analgesia and sedation after dexmedetomidine administration to cats.

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 Dxmedetomidine is an α2-adrenoceptor agonist commonly used in small animal practice to cause sedation and analgesia.1,2 In a study1 in cats, dexmedetomidine-mediated analgesia was short lasting and only evident after the administration of fairly high doses, which suggested that the clinical use of dexmedetomidine for the treatment of pain would require continuous IV infusions. In another study,4 analgesia was found to be related to plasma dexmedetomidine concentration; however, the assessment of the analgesic effect in that study was based on subjective evaluations of the response to stimuli of poorly controlled intensities. To the authors’ knowledge, the plasma concentration–analgesia and plasma concentration–sedation relationships for dexmedetomidine have not been adequately characterized in cats.

Abbreviations

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<th>ΔT</th>
<th>Temperature difference between thermal threshold and skin temperature</th>
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<td>Emax</td>
<td>Maximum effect</td>
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Thermal threshold testing has been validated and widely used in cats to study the effects of analgesic drugs.1,5–20 Results of experiments on the effects of dexmedetomidine suggest that the level of sedation does not influence the thermal threshold.1

The objectives of the study reported here were to evaluate the effect of IV administration of dexmedetomidine on thermal threshold, to characterize the relationships between plasma dexmedetomidine concentration and thermal threshold and sedation, and to design an infusion regimen that would maintain antinociceptive effects of dexmedetomidine for 6 hours. We hypothesized that dexmedetomidine would increase thermal threshold and sedation in a plasma concentration–dependent manner.

Materials and Methods

Animals—Five healthy 1- to 2-year-old spayed female cats (mean ± SD body weight, 4.7 ± 0.6 kg) were used in the study, which was conducted simultaneously...
with another study\textsuperscript{21} performed by our research group. The study was approved by the Institutional Animal Care and Use Committee at the University of California-Davis.

**Preparatory procedures**—Prior to the study, each cat was anesthetized and a vascular access port was implanted, with the catheter in a carotid artery and the port located in the subcutaneous tissues between the scapulae as described for the study\textsuperscript{21} conducted simultaneously by our research group. The port was used for collection of blood samples. Patency of the port was maintained by filling the port and catheter with heparin (1,000 U/mL) 3 times/wk. Cats were allowed to acclimate to the laboratory and to thermal threshold testing for 4 weeks before the start of the study.

**Treatment administration**—On the day of an experiment, a 22-gauge, 25-mm catheter was inserted in a cephalic vein of each cat; that catheter was used for treatment administration. Dexmedetomidine hydrochloride (5, 20, or 50 µg/kg), acepromazine maleate (0.1 mg/kg), or saline (0.9% NaCl) solution (1 mL) was administered IV as a bolus. All drugs were diluted with saline solution to a total volume of 1 mL. All cats received all treatments in a random order (determined by a computer-generated list). Each treatment was administered on a separate day, and there was at least 2 weeks between successive treatments.

**Collection of blood samples for analysis**—Methods for collection of blood samples, drug analysis, and pharmacokinetic analysis have been reported in the study\textsuperscript{21} conducted simultaneously by our research group. Briefly, blood samples were collected immediately before and at various times from 1 to 480 minutes after drug administration. Plasma was separated by centrifugation and frozen until analysis. Only plasma obtained from cats during the dexmedetomidine treatments was submitted for analysis to determine drug concentrations.

**Thermal threshold testing**—Hair on the lateral aspect of the thorax of each cat was clipped on the day before each experiment. Alternating sides of the thorax were used for thermal threshold testing for the various treatments, but the same side was used for the 8 hours after treatment administration. Dexmedetomidine was administered to each cat.

**Assessment of sedation**—Sedation was scored before each determination of thermal threshold. Sedation was scored in accordance with a scoring system.\textsuperscript{21} Briefly, scores ranged from 0 to +4 (0 = no sedation, 1 = able to stand but wobbly, 2 = sternal recumbency, 3 = able to lift head, and 4 = in lateral recumbency and unresponsive to a hand clapping). If a cat was in sternal recumbency, it was positioned in lateral recumbency. If the cat did not attempt to return to sternal recumbency, a score of 3 or 4 was assigned, depending on the response to a hand clapping. Sedation scoring was performed by the same investigator (BHP), who was unaware of the treatment administered to each cat.

**Pharmacodynamic analysis**—Plasma dexmedetomidine concentration at the time of sedation scoring and thermal threshold testing was predicted by use of individual pharmacokinetic estimates corresponding to the biexponential or triexponential model reported in the study\textsuperscript{21} conducted simultaneously by our research group. Simple and sigmoid models for the increase in ΔT or sedation score with (thermal threshold) and without (sedation) a baseline effect were fitted to the predicted plasma dexmedetomidine concentration–effect data.

For the simple Emax with baseline effect model, the following equation was used:

$$ E_c = E_0 + (\frac{E_{\max} \times C}{EC_{50} + C}) $$

where $E_c$ is the effect at plasma dexmedetomidine concentration $C$, $E_0$ is the baseline $\Delta T$ before the administration of dexmedetomidine, and $EC_{50}$ is the plasma dexmedetomidine concentration at which 50% of Emax is achieved.

For the sigmoid Emax with baseline effect model, the following equation was used:

$$ E_c = E_0 + (\gamma \times \frac{E_{\max} \times C}{EC_{50} \gamma + C}) $$

where $\gamma$ is a sigmoidicity factor (Hill's coefficient).

For the simple Emax without baseline effect model, the following equation was used:

$$ E_c = (\frac{E_{\max} \times C}{EC_{50} + C}) $$

For the sigmoid Emax without baseline effect model, the following equation was used:

$$ E_c = (\gamma \times \frac{E_{\max} \times C}{EC_{50} \gamma + C}) $$

The Emax was fixed at 4 (ie, the maximum possible sedation score) for the sedation model. The model...
with the best fit was selected on the basis of visual observation of the residuals plot and Akaike information criterion.

Infusion of dexmedetomidine to achieve 99% of Emax—The median dexmedetomidine concentration achieving 99% of the Emax on thermal threshold was calculated in the pharmacodynamic analysis. Pharmacokinetic simulations based on the mean pharmacokinetic model in the study conducted simultaneously by our research group were performed to establish an IV infusion regimen targeting the calculated median concentration that achieved 99% of the Emax. This experiment was conducted with the same 5 cats used for the 5 treatments; it was conducted within 1.5 months after completion of the previous experiments. The day before this experiment, cats were briefly anesthetized with isoflurane in oxygen. A 24-gauge, 12-cm catheter was placed in a jugular vein, capped with an infusion plug, and sutured to the skin. A 20-gauge, 5-cm catheter was placed in a saphenous vein, capped with an infusion plug, and taped to the skin. The hair on the lateral aspect of the thorax was clipped. Bandages were placed over the catheters, and cats were allowed to recover from anesthesia. The next day, thermal threshold testing was performed. Baseline thermal threshold was measured as described for the previous experiments (ie, duplicate values obtained at a 20-minute interval, with the mean calculated for the baseline value). A blood sample (2 mL) was collected from the catheter in the jugular vein and transferred to an EDTA-containing tube. A syringe and administration set were connected to the catheter in the medial saphenous vein and used to infuse a solution containing 50 µg of dexmedetomidine hydrochloride/mL in accordance with the infusion regimen determined by the pharmacokinetic simulation; the dexmedetomidine solution was infused for 6 hours by means of a syringe pump. Sedation was scored and thermal threshold determined hourly. Blood samples were collected from the catheter in the jugular vein 1, 3, and 6 hours after onset of the dexmedetomidine infusion. Blood samples were centrifuged for 10 minutes at 3,901 × g at 4°C within 10 minutes after collection; plasma was separated and stored frozen at −20°C until analysis for determination of the dexmedetomidine concentration.

Dexmedetomidine analysis—Dexmedetomidine concentration was quantified in protein-precipitated plasma samples by means of liquid chromatography–mass spectrometry performed in accordance with a previously reported technique. Detomidine-D₃ was used as the internal standard.
ternal standard. The limit of quantitation was 0.1 ng/mL, as determined on the basis of the linearity of the assay and the difference between known and measured concentrations deemed acceptable (<20%). To verify accuracy and precision of the assay, quality control samples (feline plasma spiked with known concentrations of dexmedetomidine standard at concentrations of 0.4, 5, and 30 ng/mL) were included in the analysis. Accuracy (percentage nominal concentration) ranged from 91% to 95%, 98% to 105%, and 90% to 98% for 0.4, 5, and 30 ng/mL, respectively. Intraday imprecision (percentage relative SD) ranged from 2% to 9%, 2% to 3%, and 3% to 7% for 0.4, 5, and 30 ng/mL, respectively. Interday imprecision was 2.7%, 3.4%, and 4.3% for 0.4, 5, and 30 ng/mL, respectively. Accuracy >85% and imprecision <15% were considered acceptable.

Statistical analysis—Effects of time, treatment, and the time-by-treatment interaction on ∆T were analyzed by means of a repeated-measures ANOVA. Comparisons with baseline measurements within each treatment and with saline solution at each time point were conducted by means of a Dunnett test. Effects of time, treatment, and the time-by-treatment interaction on sedation scores were analyzed after Winsorization of the data outside the 5th and 95th percentiles of the residual distribution to reduce the impact of several outliers; a mixed-model ANOVA was then used on the Winsorized sedation scores. Because the residual errors from this analysis did not conform exactly to a normal distribution, the significance reported for the ANOVA was based on a bootstrap method (with 10,000 bootstrap replicates). The correlation between thermal threshold and sedation score was analyzed by means of Spearman correlations that were stratified by treatment, and the correlation coefficient $\rho$ was reported. Normal distribution of pharmacodynamics parameters was verified by means of the Shapiro-Wilk test. Parameters for the 3 dexmedetomidine doses were compared by means of paired Wilcoxon signed rank tests. Significance was set at values of $P < 0.05$. Thermal threshold data were reported as mean ± SD, and sedation scores and pharmacodynamic parameters were reported as median and range.

**Results**

Treatment administration—Mean ± SD baseline ∆T (all cats and all treatments) was 8.5 ± 2.7°C, and mean baseline sedation score (ie, before treatment administration) was 0 in all cats for all treatments. Six

![Figure 2](image-url) Median (range) sedation score in 5 cats after IV administration of dexmedetomidine at a dose of 5 µg/kg (A), 20 µg/kg (B), or 50 µg/kg (C); acepromazine at 0.1 mg/kg (D); or saline solution (E). Sedation scores ranged from 0 to 4 (0 = no sedation, 1 = able to stand but wobbly, 2 = sternal recumbency, 3 = able to lift head, and 4 = in lateral recumbency and unresponsive to a hand clap). See Figure 1 for remainder of key.

![Figure 3](image-url) Relationship between plasma dexmedetomidine concentration and ∆T (solid line) and between plasma dexmedetomidine concentration and sedation score (dashed line) predicted by use of the median values for the pharmacodynamic parameters. Baseline ∆T (before administration of dexmedetomidine) was subtracted from the ∆T data to improve comparability of the 2 curves.
of 60 sedation scores after treatment were adjusted by Winsorization. Changes in ΔT and sedation score over time were determined for the various treatments (Figures 1 and 2). Treatment, time, and the treatment-by-time interaction significantly (P < 0.001) affected ΔT and sedation score. A significant correlation between ΔT and sedation score was found for treatment with dexmedetomidine at 5 µg/kg (P = 0.68; P < 0.001), 20 µg/kg (P = 0.78; P < 0.001), and 50 µg/kg (P = 0.64; P < 0.001). We did not detect a significant correlation (P = 0.13; P < 0.001) between ΔT and sedation score after acepromazine treatment.

We did not observe hysteresis between changes in plasma dexmedetomidine concentration and changes in ΔT or sedation score. A sigmoid Emax model best fit the plasma dexmedetomidine concentration–ΔT data and plasma dexmedetomidine concentration–sedation data. No significant difference between the model parameters obtained for the 3 dexmedetomidine doses was found; therefore, the estimates for each dose were pooled for calculation of a single summary value (Figure 3; Table 1).

Infusion of dexmedetomidine to achieve 99% of Emax—Mean pharmacokinetic parameters (A, B, and C, which are the y-intercepts of extrapolated lines for the distribution, elimination, and terminal portions of the curve, respectively; and α, β, and γ, which are the rate constants for the distribution, elimination, and terminal portions of the curve, respectively) were determined for administration of dexmedetomidine at 20 µg/kg. Simulations based on these mean pharmacokinetic parameters (A = 253 ng/mL, B = 39 ng/mL, C = 9 ng/mL, α = 0.81/min, β = 0.088/min, and γ = 0.013/min) indicated that administration of dexmedetomidine at 7.7 µg/kg over a 15-minute period, followed by administration of 10 µg/kg over a 60-minute period, and then by administration at a constant rate of 7.7 µg/kg/h would maintain concentrations above the concentration achieving 99% of the Emax on ΔT (7.6 ng/mL) beginning 2 minutes after the onset of drug administration. Plasma dexmedetomidine concentrations during administration of these infusions were measured (Figure 4). Concentrations were lower than targeted and typically increased during the infusions. Changes in ΔT and sedation score over time during the dexmedetomidine infusions were measured (Figure 5). No significant change in ΔT over time was found.

Discussion

Dexmedetomidine increased ΔT following IV administration as a bolus at all doses evaluated in the study reported here; the duration of the effect was similar and short for the 2 lower doses and longer for the largest dose. These results are partially in disagreement with those of a previous study1 in which a thermal
antinociceptive effect was found only after administration of 40 µg of dexmedetomidine/kg, whereas doses ranging from 2 to 20 µg/kg did not significantly increase ΔT. In that study,1 the drug was administered IM, whereas the drug was administered IV in the present study. Initial concentrations would be expected to be lower after extravascular administration, compared with initial concentrations after IV administration. The peak concentration after IV administration in humans was approximately 10 times as high as the peak concentration after IM administration of the same dose, and large differences in concentration were maintained for 30 minutes.24 Duration of the thermal antinociceptive effect after IM administration of 40 µg of dexmedetomidine/kg was 3 hours,1 which was similar to the duration of increase in ΔT after IV administration of 50 µg/kg in the present study.

Profound sedation was induced by IV administration of a bolus of dexmedetomidine at all doses evaluated. The duration of the sedative effect was dose dependent and equal to or longer than the duration of the thermal antinociceptive effect. This is in agreement with results of a previous study.1 The acepromazine group was included in the present study in an attempt to determine whether sedation was likely to influence thermal threshold measurement. Acepromazine is a tranquilizer that is not considered to cause analgesic effects, at least not when administered alone.25 Acepromazine did not affect thermal threshold in a previous study26 in cats. In the present study, acepromazine had a small effect on ΔT (larger than the value for saline solution at 15 minutes after administration but not different from the baseline value) and induced moderate sedation, which suggested that sedation may not interfere with thermal threshold determination. However, sedation was more profound after administration of the dexmedetomidine doses, as illustrated by the higher sedation scores; therefore, an effect of profound sedation on thermal threshold (rather than a true antinociceptive effect) cannot be excluded. Sedation and increased ΔT were evident concurrently with dexmedetomidine and therefore appeared to be closely associated, as illustrated by their significant correlation, which precluded the possibility of differentiating between thermal antinociception and a decreased response attributable to sedation. In addition, the pharmacodynamic model based on the median values for the parameters predicted nearly identical plasma dexmedetomidine concentration–effect relationships for the thermal antinociceptive and sedative effects, which added to the difficulty of separating these effects (Figure 3).

Pharmacodynamic parameters for the effect on ΔT and sedation were not significantly affected by the dose of dexmedetomidine administered. This was expected, considering that the effect appeared to be directly related to the plasma dexmedetomidine concentration and that there were initially high enough concentrations for all doses to cause effects near Emax.

The concentration achieving 99% of the Emax on ΔT was targeted for the dexmedetomidine infusion experiment. That concentration was selected in an attempt to cause the maximum antinociceptive effect of dexmedetomidine. Because the concentration-effect relationship was steep, as illustrated by the median value of 4.3 for Hill’s coefficient, the dexmedetomidine concentration achieving 99% of the Emax (7.6 ng/mL) was not much higher than other concentrations predicted to cause effects near Emax (eg, the concentration achieving 95% of the Emax [5.2 ng/mL]) and was lower than the peak concentration after IV administration of 5 µg/kg, which is a dose close to the lower end of the range used clinically in cats.

Plasma dexmedetomidine concentrations during infusion of the drug were approximately 50% of the targeted concentration, which suggested that the parameters used for the simulation were not good predictors of the disposition of dexmedetomidine. The low concentrations and the fact that they typically slowly increased throughout the infusion suggested that the values for volume of distribution and clearance used for the simulations were smaller than their actual value during the study. The effect on ΔT was highly variable and was not significant at any time; however, because the concentrations were much lower than desired, that part of the study has limited relevance.

Limitations of the present study included a small sample size, which limited statistical power for some comparisons; use of a thermal nociception model, which was not directly clinically relevant and did not allow us to detect components of analgesia other than antinociception (eg, antihyperalgesia); subjective sedation scoring; lack of profound sedation for the positive sedation control (acepromazine) treatment; and lower-than-targeted drug concentrations in the infusion experiment. In addition, effects other than thermal antinociception and sedation were not evaluated. In particular, cardiovascular effects at these doses may have been important considerations and may limit the clinical use of dexmedetomidine for analgesia.

Dexmedetomidine administered IV at doses ranging from 5 to 50 µg/kg induced thermal antinociception and profound sedation in cats. The relationships between plasma dexmedetomidine concentration and effect on thermal nociception and sedation are nearly identical, which suggested that if the antinociceptive effect is maximized, there will be profound sedation, which may not be desirable in all clinical patients. Conversely, analysis of the results indicated that if thermal antinociception is predictive of the clinical analgesic effect of dexmedetomidine, observation of profound sedation could be used as a surrogate for measurement of analgesia.

a. Provided by Orion Pharma, Turku, Finland.

References

4. Ansah OB, Raekallio M, Vainio O. Correlation between serum concentrations following continuous intravenous infusion of...