A model of transient laryngeal hemiplegia in dogs through conduction blockade of the recurrent laryngeal nerve

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OBJECTIVE
To create a model of transient unilateral laryngeal paralysis (LP) that will allow the study of cricoarytenoideus dorsalis dysfunction and a method for quantification of varying degrees of LP in dogs.

ANIMALS
5 castrated male research Beagles.

PROCEDURES
Between January and February 2018, dogs were anesthetized and instrumented with a laryngeal mask airway and a flexible endoscope to record the rima glottidis. The left or right recurrent laryngeal nerve (RLn) was localized using ultrasonography and electrical stimulation, then conduction blockade was induced with perineural lidocaine. The normalized glottal gap area (NGGA) was measured before and every 15 minutes after the block. Inspired 10% carbon dioxide (CO2) was administered for 1 minute at each sampling time. The inspiratory increase in NGGA (total and each side) was measured at peak inspiration. The change in hemi-NGGA for the control side versus the anesthetized side was evaluated with a mixed-effect model.

RESULTS
During CO2 stimulation, the increase in inspiratory hemi-NGGA was consistently less (P < .001) for the treated side (-8% to 13%) versus the control side (49% to 82%). A compensatory increase (larger than at baseline) in the control hemi-NGGA was observed. The total NGGA remained unaffected.

CLINICAL RELEVANCE
Unilateral local anesthesia of the RLn produced transient unilateral LP with a compensatory increase in the hemi-NGGA for the contralateral side. This model could facilitate the evaluation of respiratory dynamics, establishment of a grading system, and collection of other important information that is otherwise difficult to obtain in dogs with LP.
Materials and Methods

The following work was approved by the local Institutional Animal Care and Use Committee, and this report follows Animal Research: Reporting of In Vivo Experiments guidelines. Five male castrated Beagles, 4 to 5 years old, weighing 7.8 to 11.5 kg, and belonging to our institution were included in this study (study period, between January and February 2018). Dogs were fasted from solid food overnight prior to anesthesia.

Anesthesia and instrumentation

A catheter was placed in a cephalic vein, and ondansetron (0.5 mg/kg), acepromazine (0.01 mg/kg), and dexmedetomidine (1 μg/kg) were administered IV. Oxygen was supplemented via a loose-fitting face mask for approximately 3 minutes, then anesthesia was induced and maintained with a target-controlled infusion of propofol (central compartment target plasma concentrations of 5 μg/mL during induction and 3.5 μg/mL during maintenance), based on available pharmacokinetic data. Dexmedetomidine (1 μg/kg/h) and lactated Ringer solution (3 mL/kg/h) were infused IV for the duration of anesthesia. Monitoring consisted of ECG, pulse oximetry, oscillometric blood pressure measurements, rectal temperature measurements, and capnography. Dogs breathed spontaneously throughout. The airway was instrumented as described previously for laryngeal endoscopy. Briefly, a size 4 laryngeal mask airway (LMA; AES, Black Diamond) was placed orally under direct laryngoscopy. An anesthesia nonrebreathing circuit providing oxygen at 2 to 3 L/minutes was connected to the LMA through a 3-way adaptor. A 4.3-mm outer diameter flexible endoscope (Ambu View, Ambu A/S) was inserted through the free port of the adaptor and advanced through the lumen of the LMA so that the image captured both arytenoid cartilages, vocal folds, and ventral and dorsal commissures of the larynx.

Dogs were placed in lateral recumbency with the RLn to be blocked uppermost (selected at random by coin toss). A combined technique using ultrasound (Sonosite Edge, Fujifilm Sonosite Inc) with a high-frequency linear array transducer (HFL50, Fujifilm Sonosite Inc) and electrolocation (Stimuplex HNS12, B. Braun) was used. The ultrasound transducer was placed on the lateral aspect of the cranial cervical region to obtain a short-axis view of the trachea and the carotid artery (ie, transverse plane; Figure 2). An echogenic 21-gauge X 10-cm stimulating needle (MILA International, Inc) was inserted dorsal to the ultrasound transducer and advanced in an in-plane approach toward the neurovascular sheath containing the vagosympathetic trunk, RLn, and carotid artery. Needle placement was confirmed using electrostimulation (square wave current, 0.2 mA at 1 Hz) and observation of evoked responses (Supplementary Video S1). A volume of 0.3 to 1.0 mL of lidocaine 1% or 2% (AuroMedics Pharma, LLC) was injected under ultrasound guidance until the solution could be observed to dilate the perineural sheath.

Measurement of the rima glottidis and respiratory simulation

The normalized glottal gap area (NGGA) was measured as described previously. Briefly, the area delineated by the arytenoid cartilages and vocal folds, and the glottal height (vertical dotted line) is the distance between the dorsal and ventral commissures.
vocal folds was measured, and a vertical line connecting the dorsal and ventral commissures divided each hemiglottal gap area and determined the height of the glottal gap (NGGA = Glottic area/Length^2). The delineated area was normalized to the square of the glottal height, resulting in a unitless number (Figure 1). The NGGA was measured immediately prior to arytenoid abduction and at peak inspiration (maximal abduction) during 10% carbon dioxide (CO2) stimulation.

Video recordings of the larynx were obtained beginning 30 seconds prior to CO2 stimulation. CO2 was administered for 1 minute from a cylinder supplying medical-grade 10% CO2 (and 90% oxygen) connected to the respiratory breathing circuit; peak response to stimulation typically occurs shortly after 1 minute of stimulation. Maximal abduction was identified by observing the video recordings offline. Still images were obtained for measurement of the NGGA (Video Wizard, Womble Multimedia). The increases in the left hemi-NGGA, right hemi-NGGA, and total NGGA during CO2-stimulated inspiration were expressed as percent change relative to the area before inspiration (Able Image, Mu Laboratories).

Data collection began prior to local anesthesia of the RLn (baseline) and continued every 15 minutes until no asymmetry in arytenoid abduction was observed. After that point, anesthetic infusions were stopped, atipamezole (0.5 mg, IM) was administered, and dogs recovered from anesthesia.

Statistical analysis

The total duration of anesthesia, from induction to removal of the LMA, was measured. The duration of the RLn block was measured as the time from the first video with a reduction in the anesthetized hemi-NGGA until asymmetry during inspiration was no longer present. In each dog, only 1 RLn was anesthetized; hence, the contralateral side served as a control. Abduction was considered asymmetric if 1 side contributed less than 40% to the total increase in NGGA during inspiration. To account for different durations of LP, the total duration of block for each dog was normalized and expressed from 0 to 1 (time of video [minutes]/total duration [minutes]). Videos obtained at 0, 0.25, 0.5, 0.75, and 1 (or the closest fraction) were included for statistical analysis. The change in control versus anesthetized hemi-NGGA was evaluated with a mixed-effect model, with the dog as the random effect, and time and treatment (RLn block), and their interaction, as the fixed effects. Post hoc slide tests were performed at individual times, corrected for multiple comparisons. Values of $P < .05$ were considered significant. Given the small sample size, results are summarized as median and range. Statistical analysis was performed with statistical software (JMP Pro 15, SAS Institute Inc).

Results

All dogs completed the investigation without complications. Clinically normal laryngeal function was observed in all dogs before and after completion of the investigation, and no signs compatible with LP were recognized before or after the procedure. In the first 3 dogs, lidocaine 2% was used. In the last 2 dogs, lidocaine 1% was used to reduce the duration of block. Blockade of the RLn was successful in all animals; however, in 1 dog, an initial injection of 0.5 mL lidocaine 1% failed to affect arytenoid abduction, and a second injection of the same dose was administered, resulting in a block of the RLn.

Complete block (absence of ipsilateral arytenoid abduction during inspiration) was observed in all dogs 15 minutes after administration of lidocaine (Supplementary Figure S1). Median duration of anesthesia was 4.5 hours (range, 3.5 to 5.5 hours). Median duration of RLn block was 3.5 hours (range, 1.0 to 4.5 hours).

Treatment (control vs local anesthesia; $P < .001$) and the interaction of treatment and time ($P < .001$) affected the hemi-NGGA significantly. In all dogs, a reduction in the hemi-NGGA ipsilateral to the block was observed during local anesthesia, with an associated increase in hemi-NGGA for the contralateral side. The total NGGA remained unchanged. These changes were observed at all times except baseline and recovery (Figure 3).
Discussion

In this investigation, we describe a model of transient unilateral LP in dogs, induced by the administration of lidocaine perineural to the RLn. Although LP developed quickly in all dogs, recovery occurred progressively, allowing measurements during varying degrees of partial unilateral block. Laryngeal endoscopy coupled with respiratory stimulation with CO$_2$ was used to quantify those changes.

Perineural injection of lidocaine blocked conduction consistently, resulting in ipsilateral immobile arytenoid cartilage. In this initial investigation, lidocaine was injected under ultrasound guidance until the solution could be observed to dilate the perineural sheath. As a result, different volumes were used, and a large variability in the duration of block resulted. A standardized volume of injection is likely to reduce this variability. Blockade of very short duration will result in fewer opportunities to measure partial degrees of arytenoid dysfunction; thus, sufficient duration of block to capture varying degrees of LP might be desirable. For all dogs, clinically normal function was restored within 4.5 hours.

The model we present here might be useful to investigate the consequences of varying degrees of partial LP on particular aspects of respiratory function, such as the magnitude of thoracic negative pressure at peak inspiration, upper airway resistance, and work of breathing. With the use of respiratory stimulants, such as CO$_2$ or doxapram, these measurements can be obtained at maximum effort. The information obtained might also reveal characteristics that can be used to diagnose LP at early stages in predisposed breeds, or during surgeries that could compromise the integrity of the RLn. For example, measurement of partial deficiencies in arytenoid abduction could be used to screen dogs at risk prior to any report of signs of LP. We have recently reported a case in which subclinical LP was identified using this methodology, and its progression quantified, in a Beagle. LP results in slow but progressive atrophy of the CAD muscles, decrease in fiber diameter, loss of muscle fibers, fibrosis, and replacement of muscle fibers with fat, secondary to denervation. Although surgical reinnervation procedures have been described in people, success depends, at least in part, on the presence of viable CAD muscles and RLn if nerve-to-nerve anastomosis is performed. Thus, early identification of affected individuals may be important if treatment is to be instituted with the most viable possible CAD muscle.

Previous grading schemes for canine LP have used unilateral, bilateral, partial, and complete LP criteria. Quantification of laryngeal abduction resulting from a standardized stimulus could be useful for generating a grading system that describes the magnitude of dysfunction, equivalent to those used to grade dysfunction in horses at rest and under maximal exercise stimulation. The methods described might be useful to quantify the degree of dysfunction in dogs with naturally occurring LP. This technique could be applied not only to grade the severity of the disease but also when assessing early-stage LP and its progression, and investigating whether the speed of progression has prognostic value. Quantification might also serve to evaluate the recovery of function with future potential therapies. The use of this methodology for grading cases of bilateral dysfunction may require additional information because a control inspiratory hemi-NGGA is not available. It is possible, however, that a normal total inspiratory NGGA can be used as a reference. Such a reference will most likely need to be obtained with a standard anesthetic technique and respiratory stimulation, and interbreed differences should be evaluated.

For our study, we chose to use CO$_2$ as the respiratory stimulant. CO$_2$ administered through an LMA for 1 minute every 15 minutes produced a reproducible response. This was important because repeated respiratory challenges were needed to assess the changing degrees of LP during recovery after local anesthetic block of the RLn. Recently, it has been shown that the laryngeal response to 10% CO$_2$ is similar to that of doxapram (0.5 mg/kg IV), but the CO$_2$ challenge produced less tachycardia and hypertension than the injectable stimulant. A higher dose of doxapram, 2.2 mg/kg, resulted in an even greater magnitude of laryngeal abduction, but with more severe hypertension and cardiac dysrhythmias. Thus, CO$_2$ might be a suitable stimulant when repeated challenges are needed.

Our results indicate that, despite a marked decrease in the inspiratory NGGA on the anesthetized side, a compensatory increase in NGGA during inspiration was observed on the contralateral side. As a result, the total NGGA during inspiration was not altered substantially. A similar observation was reported previously in horses over a longer period of time following injury of an RLn and in a dog with subclinical left-side LP. The compensatory increase in arytenoid abduction on the uninjured side probably mitigates the effects of unilateral LP until paradoxical adduction occurs, or until the disease progresses on the contralateral side.

There are limitations to our work. The sample size was small and the dose of lidocaine administered perineurally was not standardized. Thus, conclusive evidence on the speed of onset or duration of block of the RLn should not be drawn from these data. Because of the variation in the duration of RLn dysfunction, we normalized the duration to facilitate statistical analysis. It was possible that this step could be avoided if a standardized dose of lidocaine was used, resulting in a more uniform duration of action. The sample size was also too small to draw conclusions regarding the possibilities of complications from this technique. However, this proof-of-concept investigation supported the proposition that local anesthesia of the RLn could serve as a model of LP and could provide important information that is otherwise difficult to obtain. More work in this area may help us determine the optimal dose of lidocaine and to obtain more data on the consequences of LP on respiratory mechanics. Our data were obtained from...
dogs anesthetized with propofol and dexmedetomidine, and results to respiratory stimulation could vary if other anesthetics or stimulants are used. Although the ultrasound-guided block provided a minimally invasive method to produce transient LP, and no complications were observed, the technique is not risk free; inadvertent intravascular injections are possible as well as nerve trauma.

In summary, local anesthesia of the RLn resulted in ipsilateral, transient, paralysis of the CAD muscle and absence of inspiratory arytenoid abductor. A compensatory increase in hemi-NGGA was observed for the contralateral side, which maintained the total NGGA unaffected. Local anesthesia of the RLn may serve as a model to study LP in dogs.

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Supplementary Materials
Supplementary materials are posted online at the journal website: avmajournals.avma.org