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Myotonic Myopathy in a Miniature Schnauzer: Case Report and Data Suggesting Abnormal Chloride Conductance Across the Muscle Membrane

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A 1-year-old 6.0-kg, sexually intact male Miniature Schnauzer was examined due to difficulty swallowing, increased upper respiratory sounds, ptyalism, difficulty rising, and a stiff gait. Physical examination revealed enlarged skeletal muscles that were painless when palpated. Decreased tongue mobility was noted; the tongue appeared wide at the base. Inspiratory stridor was present and paralysis of the vocal folds was noted during laryngeal examination under light sedation. The dog showed considerable difficulty rising from a sternal position and required approximately 45 seconds until it could stand and walk. The dog had little difficulty walking but appeared stiff and exhibited a "bunny-hop" in its hind limbs when running. The dog was unable to step onto a 4-inch platform, and when placed on the platform appeared unable or unwilling to step down. No dimpling of the appendicular muscles was noted after percussion. Lateral compression of the tongue caused a noticeable dimple that persisted for 30 seconds after pressure was removed. A myopathy was suspected. Differential diagnoses included myotonic myopathy, muscular dystrophy, myositis, lysosomal storage disease, or other inherited myopathies.

A CBC and serum chemistry analysis were unremarkable. A serum creatine phosphokinase concentration was 466 U/L (reference range, 25–467 U/L). A urine cortisol to creatinine ratio was 14.3 (reference range, < 35). An antinuclear antibody titer and *Toxoplasma* titer were negative. A urine metabolic screen, testing for abnormal substrates of metabolism, revealed no evidence of a primary metabolic disease. Thoracic radiographs, electrocardiography, and cardiac ultrasound revealed no cardiac abnormalities.

Electrodiagnostic testing was performed on the dog after induction of anesthesia with 15 mg/kg IV Pentothal (thiopental sodium, Abbott Labs, North Chicago, IL) and 0.5 mg/kg diazepam (Steris Labs, Phoenix, AZ), tracheal intubation, and maintenance of anesthesia with inhalant isoflurane. While anesthetized, the rectal temperature of the dog was maintained between 99°F and 100°F. Electromyographic examination was recorded using a 20-mm, 26-gauge concentric needle electrode inserted into the muscle and a digital amplifier (DANTEC, Skovlunde, Denmark). Amplifier filters were 5 kHz to 5 Hz; responses were recorded at a sensitivity of 100 μV/cm and a sweep speed of 10 milliseconds/cm. Myotonic discharges, sounding like several revving motorcycles, were recorded from all muscles examined including the appendicular muscles, epaxial muscles, muscles of the head and neck, laryngeal muscles, and tongue. These discharges waxed and waned; their frequency and amplitude decreased over time with the discharges ceasing altogether within 25 seconds. Discharges from the gastrocnemius muscle are illustrated in Figure 1 (sensitivity = 0.5 mV/cm, sweep speed = 1 second/cm). The temperature of the gastrocnemius muscle was 98.5°F, as measured by an intramuscular temperature probe (Yellow Springs Instruments Co, Inc, Yellow Springs, OH). Cooling the gastrocnemius muscle in an ice wrap to an intramuscular temperature of 86°F caused the duration of the myotonic discharges to increase to 45 seconds after needle insertion. Nerve conduction velocity testing was performed using 12-mm, 20-gauge recording electrodes inserted in an interosseous muscle and stimulating the tibial–sciatic nerve at the tarsus and ischiatic notch in the hind limb, and stimulating the ulnar nerve at the elbow and carpus in the forelimb. Stimulus duration was 200 microseconds, filter settings were 2 kHz to 20 Hz, sensitivity was 2 mV/cm, and sweep speed was 2 milliseconds/cm. Motor nerve conduction velocity of the tibial–sciatic nerve (65 m/second) and the ulnar nerve (62 m/second) were within normal limits.[1] Repetitive stimulation at 3 Hz of the ulnar nerve while recording from the interosseous muscle caused no decremental response. Sensory nerve conduction velocity of the radial nerve (200 evoked responses averaged; filter = 2 kHz to 20 Hz; stimulus rate = 10 Hz; sensitivity = 5 μV/cm; sweep speed = 2 milliseconds/cm) was within normal limits (72 m/second).[1]

Intravenous procainamide hydrochloride (Abbott Labs) was administered at 100 mg, and then at 50 mg increments over 1 hour. No change was discernible in the intensity or duration of the myotonic discharges until a total of 250 mg (41.7 mg/kg) procainamide was administered, when the amplitude of the initial activity decreased by 25% and the duration of the discharges decreased to 10–12 seconds. Serum concentrations of procainamide are listed in Table 1. The QRS, QT, and PR intervals remained within normal limits until 200 mg (33.3 mg/kg) procainamide was given intravenously. The QT interval was prolonged to 280 milliseconds after 200 mg was administered, and to 300 milliseconds after 250 mg intravenous procainamide was administered. The QRS interval increased to 56 msec after 250 mg procainamide (41.7 mg/kg) was administered. No changes in heart rate, mean arterial pressure, systolic pressure, or diastolic pressure were noted during IV procainamide administration.

A biopsy of the biceps femoris muscle stained with he-

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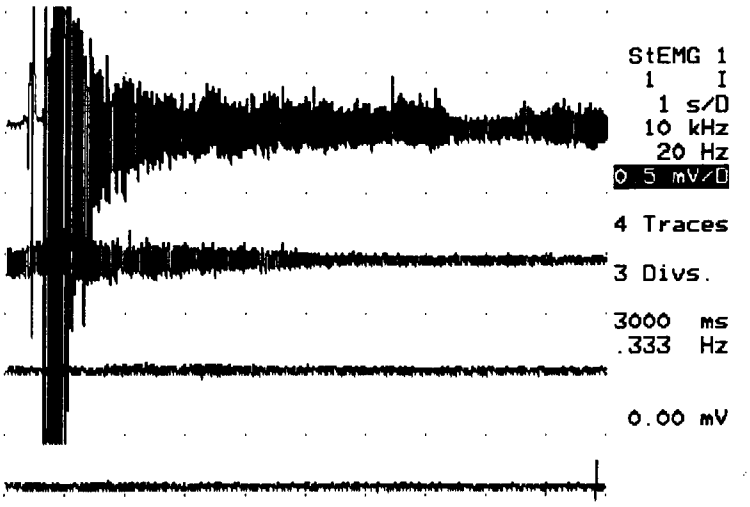

Fig 1. Electromyographic recording of the decrement in myotonic potentials over time in the gastrocnemius muscle of a dog with myotonic myopathy. Muscle temperature = 98.5°F; sensitivity = 0.5 mV/cm; sweep speed = 1 second/cm.

matoxylin and eosin revealed occasional fibers with internal nuclei. Based on these clinical, electrophysiologic, and histologic findings a diagnosis of myotonic myopathy was made.

Treatment with oral procainamide hydrochloride (Schein Pharmaceuticals Inc, Florham Park, NJ) was begun. A dosage of 240 mg (40 mg/kg) procainamide every 6 hours resulted in significant improvement in clinical signs. The dog could rise easily, its gait was less stiff, abnormal upper airway sounds resolved, and its ability to eat and drink improved markedly. The dog was able to step onto a 4-inch platform yet was unable to walk either up or down a flight of stairs. Following 1 week of therapy, the serum procainamide concentration was 10.0 μg/mL 2 hours after oral dosing, 9.9 μg/mL 3 hours after dosing, and 8.4 μg/mL 6 hours after oral dosing. After 3 weeks of 240 mg oral procainamide every 6 hours, a CBC, serum chemistry analysis, and electrocardiogram revealed no significant abnormalities. Doses of procainamide less than 240 mg produced less improvement in clinical signs; doses of 300 mg or higher produced weakness and lethargy within 2 hours of dosing that resolved by the 3rd hour. A significantly greater serum concentration was necessary to alter spontaneous electrical activity than was necessary to cause improvement in clinical signs.

Oral procainamide was discontinued, and oral mexiletine hydrochloride (Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT) was administered. Two weeks of 50 mg (8.3 mg/kg) mexiletine every 8 hours resulted in a significant improvement in clinical signs; however, the improvement was no greater than that noted with procainamide. A mexiletine serum concentration, 3 hours after dosing, was 1.4 μg/mL.

Table 1. Serum procainamide concentration after intravenous procainamide administration.

	Total Dose Administered (mg)			
	100	150	200	250
Serum concentration (μg/mL)	34.0	24.8	40.5	54.5
Improvement in electromyographic abnormality	No	No	No	Yes

Myotonia may result from defective chloride or sodium conductance across the muscle membrane.[2] Defects in chloride conductance cause myotonia by resulting in a high specific membrane resistance.[2] A simple in vitro electophysiologic experiment was performed to determine whether specific membrane resistance was abnormally increased in the affected dog. A glass microelectrode was used to deliver a hyperpolarizing constant-current square-wave pulse into an external intercostal muscle fiber and the input resistance (R_{in}) of the fiber was determined. By determining the mean circumference and area of the muscle fibers within the intercostal biopsy, the mean membrane resistance for unit area of fiber surface (R_m) was determined by the formula[3]:

$$R_m = \frac{(R_{in})^2(\pi r^2)(2\pi r)}{(0.25)(185\ \Omega cm)}$$

where r is the radius and 185 cm is an estimate of the specific axoplasmic resistance.[4]

The myotonic dog and 2 unaffected mongrel dogs were induced with thiopental and maintained under anesthesia with isoflurane. External intercostal muscle was excised from the 7th–8th intercostal space, making certain that the muscle contained tendon at both ends. The biopsy was placed in oxygenated phosphate-buffered saline and transported to the electrophysiologic recording chamber within 15 minutes. The external intercostal muscle biopsy was pinned at resting length and continuously perfused with a solution containing 108 mmol/L NaCl, 3.5 mmol/L KCl, 1.5 mmol/L $CaCl_2$, 0.7 mmol/L $MgSO_4$, 26.2 mmol/L $NaHCO_3$, 1.7 mmol/L NaH_2PO_4, 9.6 mmol/L sodium gluconate, and 5.5 mmol/L glucose. The solution was vigorously aerated with 95% O_2 and 5% CO_2. Recordings were performed at room temperature (18–22°C). Microelectrodes were filled with 3 M KCl, and only those with resistances between 5 and 7 megaohms were used. A single electrode was used to give a 10-nA hyperpolarizing pulse lasting 100

milliseconds. This allowed the membrane potential to reach steady state during the pulse. No voltage offset occurred at the beginning of the current pulse. The membrane potential was recorded before and during the current pulse. The same electrode was used to pass current and to record the change in membrane potential using a Model 1600 Neuroprobe amplifier (AM Systems, Washington, DC). The offset in membrane potential was used to calculate the input resistance using the equation V = IR, where V = the offset in membrane potential; I = 10 nA current; and R = input resistance.[3,5] Ten to 20 muscle fibers were examined in each preparation; only fibers with a resting membrane potential of at least −50 mV or more negative were examined.

Insertion of the glass microelectrode into a myotonic fiber caused repetitive firing of the fiber that subsided within 15 seconds. The R_{in} of the intercostal muscle from the myotonic dog was 0.98 ± 0.29 megaohms. The R_{in} of the intercostal muscle from the nonmyotonic dogs was 0.46 ± 0.19 megaohms and 0.28 mV ± 0.035 megaohms.

At the time of muscle biopsy, a 2nd intercostal muscle section was snap-frozen in isopentane precooled in liquid nitrogen, cryosectioned, and stained with hematoxylin and eosin. At a magnification of 500× an image of muscle in cross-section was digitized and the area and circumference of 20 fibers was measured using a tablet pen (Jandel Scientific, San Rafael, CA) and computer monitor to outline the perimeter of muscle fibers. A computer program was developed to determine the circumference and area. The mean fiber circumference and standard deviation of the myotonic dog was 181 ± 29.5 μm; the mean muscle fiber area was 2,108 ± 653 μm^2. The mean fiber circumference of the 1st unaffected dog was 135 ± 14.6 μm, and of the 2nd unaffected dog was 130 ± 18.8 μm; the mean muscle fiber area of the 1st unaffected dog was 1,234 ± 310 μm^2 and of the 2nd unaffected dog was 1,135 ± 339 μm^2. Calculated R_m for muscle fiber from the myotonic dog was 7,849 cm^2. Averaged R_m for the 2 nonaffected dogs was 504.5 cm^2.

Myotonia is a clinical sign characterized by delayed relaxation of skeletal muscle after contraction associated with persistent repetitive electrical activity in the involved muscle.[2] Myotonia may result from a defect in either chloride or sodium conductance,[2] and mutations within the skeletal chloride and sodium channels have been identified as the cause of many of the various forms of myotonia in humans.[2,6] Myotonia may also result from the administration of hypocholesterolemic drugs in humans,[2] and from excessive circulating steroid or certain pesticides in dogs.[7] No evidence was found of excessive circulating steroid in the myotonic dog and the dog had no history of toxin exposure.

The very large R_m in the myotonic dog, when compared to the R_m of the nonmyotonic mongrel dogs, suggests a drastic reduction in resting membrane conductance. In mammalian muscle fibers, 70–80% of total resting membrane conductance is due to chloride ion conductance, and potassium ion conductance accounts for the remaining 20–30%.[2,6] Therefore, a large decrease in resting membrane conductance is only possible if a concomitant decrease occurs in chloride conductance[2]; this can be determined by calculating the membrane resistance per unit area of fiber surface.[4,8] The R_m in this myotonic dog is similar to that found in external intercostal muscle of humans with myotonia congenita and is slightly higher than that found in myotonic intercostal fibers from goats.[8] Although the R_ms of muscle fibers of nonmyotonic dogs are lower than those calculated from external intercostal muscle of normal humans (2,200 ± 223 cm^2) and goats (1,897 ± 86 cm^2),[8] they are similar to that determined in rat muscle.[4] The low R_m in our nonmyotonic dogs may reflect differences between species[8], sampling error due to the small number of nonmyotonic dogs and muscle fibers examined, or differences in the techniques used to determine the R_m.

The purpose of this paper was to present preliminary data on a defect in chloride conductance in a dog with myotonic myopathy. It must be noted, however, that the 2-electrode technique that is the standard for recording membrane physiology was unavailable to the authors, who used a single-electrode recording technique. The goal was to determine whether a significant difference in R_m occurred between normal dog muscle and muscle from the dog with myotonic myopathy. Using the same single-electrode technique for both the affected and unaffected dogs, a profound difference in R_m was identified. Rather than pursue more precise electrophysiologic evaluation of chloride conductance in the muscle membrane using a 2-electrode technique, the canine chloride channel is being cloned and evaluated to screen for abnormalities.

Tests to identify abnormalities in sodium ion conductance were not performed. Electrophysiologic testing for abnormalities in sodium channel conductance require patch clamp or 3-electrode voltage clamp studies; this equipment was unavailable. However, a sodium channel defect is unlikely to be concomitantly present in this dog because, in humans, the sodium channel is encoded by a different gene than the chloride channel.[6] However, a defect in sodium conductance possibly also exists in this dog.

A significantly larger muscle fiber circumference and area was found in the myotonic dog when compared to the nonmyotonic dogs. This increase in fiber size is also described in myotonic humans and goats[8] and may result from isometric contraction due to the myotonia,[8,9] or from differences in size of muscle fibers between different dog breeds. No significant difference in weight was found between the Miniature Schnauzer and the mongrel dogs examined.

Finally, this report documents the serum concentrations of 2 drugs used in the treatment of myotonia. Unfortunately, no effective and safe drugs exist that act directly on the chloride channel.[2] Because repetitive activation of the sodium channel is thought to be the underlying cause of the repetitive electrical activity in all forms of myotonic muscle,[2] therapy is directed at sodium channel blockade. Local anesthetic drugs in the class 1 antiarrhythmic category affect sodium channel activation and decrease net sodium influx during depolarization.[2] Procainamide (a class 1A antiarrhythmic) and mexiletine (a class 1B antiarrhythmic) have both been used in the treatment of myotonia in human patients.[2] In the myotonic dog, clinical signs markedly improved with a serum procainamide concentration of 10 μg/mL. However, electromyographic abnormalities did not improve until a serum concentration of 54.5 μg/mL was

achieved, and this was accompanied by abnormalities on the electrocardiogram consistent with those noted with increasing procainamide concentrations.[10] The electromyographic abnormalities noted in the presence of 10 μg/mL procainamide may explain why clinical signs did not completely resolve in this dog when treated with oral procainamide. A serum mexiletine concentration of 1.4 μg/mL was found to result in similar improvement but not resolution of clinical signs. Class 1B antiarrhythmics have minimal effects on cardiac impulse generation and propagation.[10] Higher doses of mexiletine were postulated to possibly result in the further improvement in clinical signs, without signs of electrocardiographic abnormalities. However, the concern of the owner about administering a drug infrequently used in veterinary medicine limited its use in this dog.

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