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TWITCH RESPONSE IN THE CANINE VOCALIS MUSCLE

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The twitch response of the canine vocalis muscle was investigated through a series of experiments conducted in vitro. Samples of vocalis muscle were dissected and prepared from canine larynges a few minutes before death and kept in Krebs solution at a temperature of 37 ± 1°C and pH of 7.4 ± 0.05. Field stimulation with parallel-plate silver electrodes was applied to study the twitch response of muscle samples. The peak tension and time course of isometric contraction of isolated muscle samples were measured electronically with a Cambridge Technology Dual Servo System (ergometer). Contraction time and 50% relaxation time of this muscle were measured for seven samples at various levels of strain. It was found that contraction time ranged between 22 and 32 ms and 50% relaxation time ranged between 17 and 37 ms. Results indicate that the vocalis muscle is a fast muscle capable of performing rapid maneuvers in support of changes in fundamental frequency.

The vocalis muscle is an intrinsic laryngeal muscle that is involved in the control of fundamental frequency, intensity, and register in phonation (Atkinson, 1978; Baer, Gay, & Niimi, 1976; Hirano, Ohala, & Vennard, 1969; Shipp & McGlone, 1971). In fundamental frequency control, the vocalis muscle serves as an antagonist to the cricothyroid muscle in regulating the length of the vocal folds. Actively shortened vocal folds generally produce lower fundamental frequencies (Hollien, 1960) because the nonmuscular tissue layers (the lamina propria, which are medial to the vocalis muscle) are more lax than in elongated folds. Because most of the vibration is confined to these nonmuscular layers, their effective stiffness largely determines the fundamental frequency. Even though the vocalis muscle may stiffen during contraction, a reduced length of the vocal folds may decrease the fundamental frequency. Only when the length is maintained constant (or increased) by simultaneous action of the cricothyroid muscle is stiffening of the vocalis likely to contribute to fundamental frequency increase.

In control of vocal intensity and register, the vocalis muscle serves as an adductor (Hirano, Vennard, & Ohala, 1970). By decreasing the vocal fold length, the cross section of the muscle increases, which reduces the prephonatory gap (glottis) between the folds. This, in turn, can result in increased glottal resistance and in vocal fold vibration (Shipp & McGlone, 1971).

Anatomically, the vocalis muscle is the medial portion of the thyroarytenoid muscle. Although there is still some uncertainty about the division of the thyroarytenoid muscle into medial and lateral (thyromuscularis) portions on a functional basis, it has not been difficult to separate them in dissection (Perlman, Titze, & Cooper, 1984). The fibers are longitudinal (anterior-posterior) in the vocalis but are oriented somewhat obliquely in the thyromuscularis (Faaborg-Andersen, 1957).

In speech and singing, it is sometimes necessary to make rapid adjustments in intensity, fundamental frequency, or vocal register. A stressed syllable, a gliding tone on a monosyllabic word, a rapidly sung scale or trill, and a transition from chest voice to falsetto (as in a yodel) are examples of such rapid adjustments. Sundberg (1979) reported response times for rising and falling pitches for men and women who were trained and untrained vocalists. Intervals of 4 to 12 semitones in pitch were achieved at an average maximum rate of about 70 ms. Trained subjects were able to execute pitch rises at an average of 60 ms, whereas untrained subjects required an average of 80 ms. On the same pitch rise maneuvers, women were about 10 ms faster than men. On pitch falls, however, the performance of untrained subjects was nearly equal to that of the trained subjects, with average response times of 60 ms. Women were again about 10 to 20 ms faster than men. Interestingly, the size of the pitch interval did not greatly affect the speed of the change. Twelve-semitone changes required only 5 to 10 ms more time than four-semitone changes in most cases.

Given that the vocalis muscle may be maximally involved in pitch lowering, it appeared to us that the approximate 60-ms limit in the speed of pitch lowering may be related to the twitch response of the vocalis muscle plus the mechanical response time needed for changing vocal fold length under nonisometric conditions. We don't know how much of that time is used in relaxation of the cricothyroid muscle (if it is active at the higher pitch) or other movement-related response times, but it is conceivable that an appreciable fraction of the approximately 60 ms is simply the vocalis twitch response time.

Martensson and Skoglund (1964) reported contraction times of the dog vocalis in isotonic twitch at 10 to 17 ms and the cricothyroid at 30 to 40 ms. Hirose, Ushijima, Kobayashi, and Sawashima (1969) measured the contraction time for the cat vocalis muscle in isometric conditions at 21 ± 5.3 ms, and for cricothyroid at 44 ± 7.2 ms. Hast (1967) reported contraction times of 14 ms and 22 ms for the dog and cat vocalis, respectively. Larson and Kempster (1985) looked at these parameters by comparing the activity of a single motor unit with changes in fundamental frequency, and reported values of 9 to 20 ms for the human vocalis and 9 to 57 for the human cricothyroid.

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It appears that there are some discrepancies in the reported data. It seems appropriate, therefore, to inquire about the active twitch response of the muscle. In particular, it may be hypothesized that the twitch response is a function of vocal fold length, and that this was not controlled in the previous studies.

The following specific questions are asked in this investigation:

1. What is the response time between a stimulating pulse and the twitch tension produced as a function of vocal fold length?
2. What is the ratio of maximum-active to passive muscle tension in twitch as a function of length?

The measurements that can answer these questions are needed to refine biomechanical computer simulation models of phonation. Because force-elongation measurements of laryngeal tissue are impossible to make on humans, we have chosen to study the dog as a model. Furthermore, for optimal control of tissue sample geometry, we have opted for an in vitro technique, to be described presently.

METHODS

Material

The samples used in this study were prepared from seven canine vocalis muscles. Due to the differences in the animals' size, age, sex, and breed, dissected samples had different dimensions (length and diameter). Those dimensions were recorded and used in the later analyses, but no effort was made to study the vocal fold twitch characteristics as a function of animal's size, age, sex, or breed.

Surgical Method

The larynx was excised a few minutes before death and immediately submerged in an aerated Krebs solution. The solution was continuously aerated with a mixture of 95% oxygen and 5% carbon dioxide. The pH of the solution was 7.4 ± 0.05, and the temperature was maintained at 37 ± 1°C with an immersion circulator (Fisher Circulation Model 73).

All dissection was performed with the larynx submerged in the aerated Krebs solution. Extrinsic muscles were removed from the thyroid cartilage, and all structures above the level of the ventricles of Morgagni were cut away, thus exposing the true vocal folds.

The thyroid cartilage was hemisected at the anterior notch, and a rectangular portion of the thyroid cartilage remained attached to the vocal fold. A similar attachment was retained at the arytenoid cartilage posteriorly, thus isolating the true vocal folds. Using a dissecting microscope, the epithelium and lamina propria were dissected from the underlying vocalis muscle. Once the vocalis muscle was exposed, muscle fibers were carefully trimmed away until the sample width was approximately 4 mm, and the depth was approximately 2 mm. A 3-0 Tevdek polyester suture was inserted through each cartilage and tied. The suture attached to the arytenoid cartilage was secured to a base of a water-jacket organ bath chamber containing aerated Krebs solution. The suture attached to the thyroid cartilage was tied to the ergometer arm (Figure 1).

Tubocurarine chloride was added to the Krebs solution in a ratio of 3 mg per 1 ml of solution to block the neuromuscular junction (Close, 1981).

Experimental Method

The displacement of the ergometer arm and the force exerted by the tissue were measured electronically with a Dual Servo ergometer. The analog force signal of the ergometer was sent to an A/D converter and computer for digital recording of the data. The position and force values were also monitored through a data logger. The sample was subjected to an initial force of about one gram to keep it straight and remove the slackness in the sutures (Alipour-Haghighi & Titze, 1985). Then the average length of the sample was measured in the chamber. The cross-sectional area was calculated from the mass of the sample after the experiment. A pair of parallel-plate silver electrodes was mounted on the chamber so that the locations of the electrodes could be adjusted for optimum field stimulation (Figure 1). The stimulation was applied with a Grass S-88 stimulator transverse to the muscle fibers.

The isolated muscle was stimulated with a single, brief electrical current (a square pulse), and the twitch response force of the muscle was digitized and recorded by computer. Because the contraction and subsequent relaxation happens very rapidly, an analog-to-digital conver-

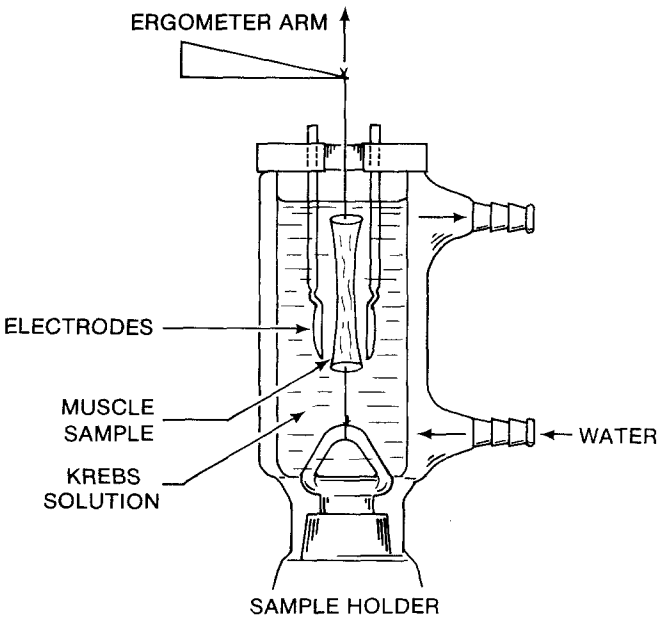

FIGURE 1. Sample mounting and electrode position.

sion rate of 20 kHz was used. We allowed the tissue samples to contract in an isometric mode, where the muscle exerts its force against an infinite load (theoretically). In other words, there was no external shortening. Electrical field stimulation was used because the stimulating current could be applied uniformly to the tissue. This is a practical way of activating an isolated muscle in vitro without damaging the fibers. To ensure uniformity of the field and complete penetration through the muscle, we trimmed the muscle fibers to a relatively small cross-sectional area. However, this trimming and reducing of the number of fibers is not only difficult, it reduces the peak active tension, making measurement error more likely. Samples with average diameters of 2 to 4 mm were used in this study. Reducing the thickness of the sample has another advantage, namely, a better definition of sample length. In the whole muscle, the length of the vocalis can vary 3 to 4 mm, due to the curvature of the thyroid cartilage, causing a decreasing distance between thyroid and arytenoid cartilages in the lateral direction.

We noticed that the amount of penetration of the field into the muscle depends not only on the amplitude and duration of the pulse, but also on the distance between the electrodes and the size of the electrodes. We therefore made the electrodes large enough to cover the whole cross section of the sample and kept them as close to the cartilages as possible without actually touching them (about 6 to 8 mm separation, Figure 1). The amplitude of the pulse had to be high enough to depolarize each muscle fiber membrane. As this amplitude was gradually increased, more muscle fibers were depolarized, and the peak of the active tension increased until all the fibers were activated. Then no further increase was observed in the peak value (saturation). Increase in pulse duration also saturated the force. For most samples, a pulse duration of 2 to 2.5 ms was best, but saturation voltage was highly dependent upon the size of the sample and the distance between the electrodes, varying between 65 and 90 volts.

RESULTS

Figure 2 is an illustration of data associated with a typical twitch response of the vocalis muscle in the saturated state, together with a stimulus signal. Three major characteristics of twitch response, namely, peak of tension, contraction time, and 50% relaxation time are denoted by F, C, and R respectively. We found the contraction time to be 22 to 32 ms, with an average of 25.7 ms and a standard deviation of 2.9 ms, and the 50% relaxation time to be 17 to 37 ms, with an average of 25.2 ms and a standard deviation of 5.6 ms.

Although total relaxation requires on the order of 100 ms, complete restoration of the distribution of calcium after contraction might take several seconds (Carlson & Wilkie, 1974). Thus, after each stimulus, we allowed the tissue to relax for about 5 min before continuing with another stimulus.

Figure 3 illustrates contraction times of samples of vocalis muscle as a function of strain. While there is variability of the data across the samples, there is not any particular trend along the strain axis. In other words, contraction time seems to be independent of elongation.

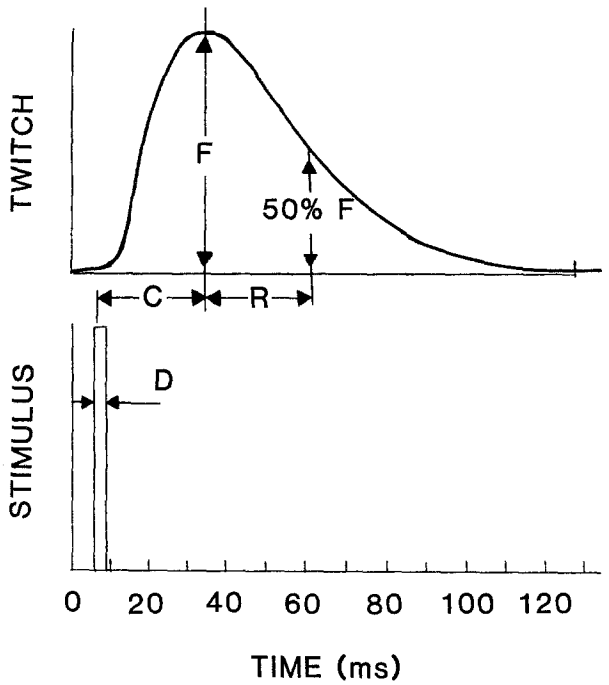

FIGURE 2. Time course of isometric twitch. F is the peak of tension, C is the contraction time, R is 50% relaxation time, and D is the stimulus duration.

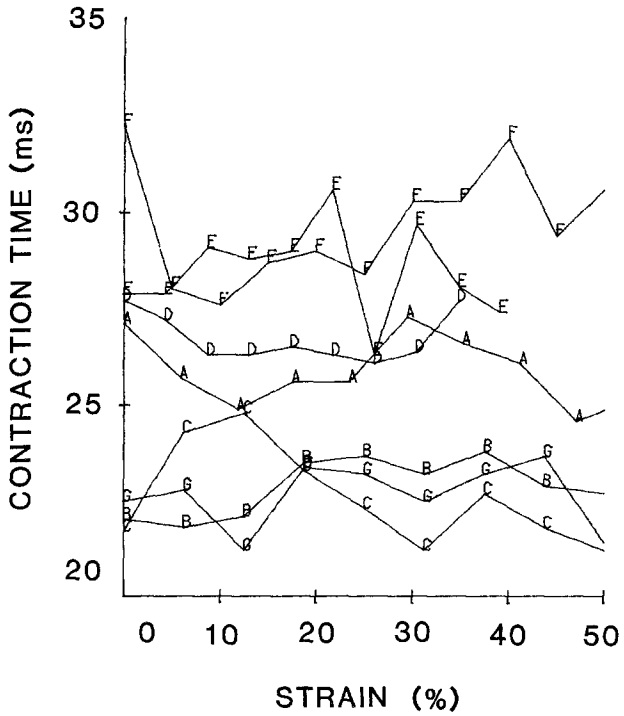

FIGURE 3. Contraction times of different samples of vocalis at various levels of strain.

Higher values of contraction (denoted by E and F) were noticed to be associated with tissue from larger animals.

Figure 4 illustrates the 50% relaxation time (or period in which tension drops to 50% of its peak value) for various samples as a function of strain. These data also show variability across samples and levels of strain, but there is also a slight increasing trend in relaxation time with strain.

Figure 5 is an illustration of typical force-elongation curves for one muscle sample. The force in question is the peak of the twitch response. The curve labeled "total" is the sum of the passive force and active force, which was computed after stimulation of the sample. The curve labeled "passive" is the force measured before stimulation of the sample. Thus, active response is calculated as the difference between total and passive force. The passive component gets progressively steeper at larger elongations, indicating more stiffness at higher strain. However, the active response behaves differently. Up to a certain point, the active response rises a little bit, then starts falling. In this sample, active response reached its peak at about 10% strain. The drop in active response could be explained by the sliding-filament model of muscle contraction. Stretching a muscle decreases the amount of overlap between actin and myosin filaments and results in lower tension.

Because in our previous study (Alipour-Haghighi & Titze, 1985) we modeled the passive elasticity of this muscle, it would be helpful if we could establish a relation between active and passive forces. This can be done by computing the active-to-passive force ratio at various levels of strain. Figure 6 illustrates the active/passive ratio for five samples. These data were obtained based on the fact that the minimum passive force of one gram corresponds to zero strain baseline. Symbols stand for data points and the solid line for an average result.

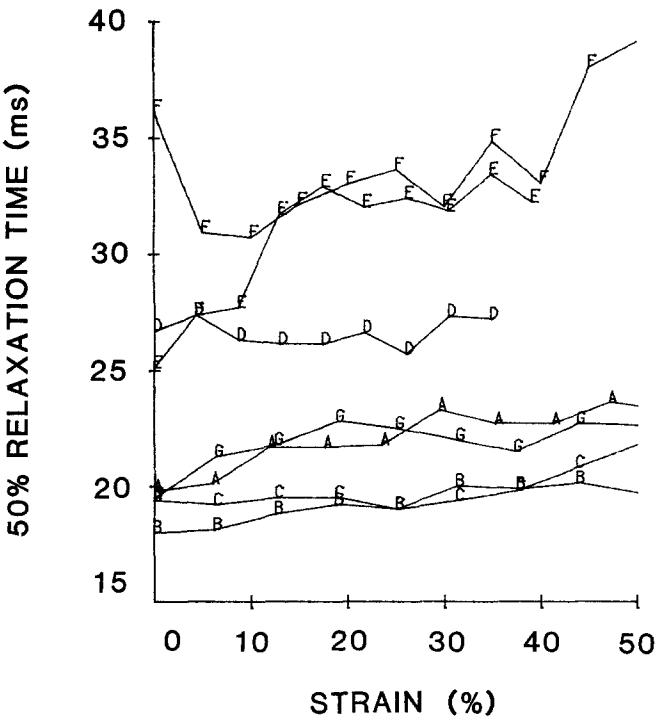

FIGURE 4. 50% relaxation times of different samples of vocalis at various levels of strain.

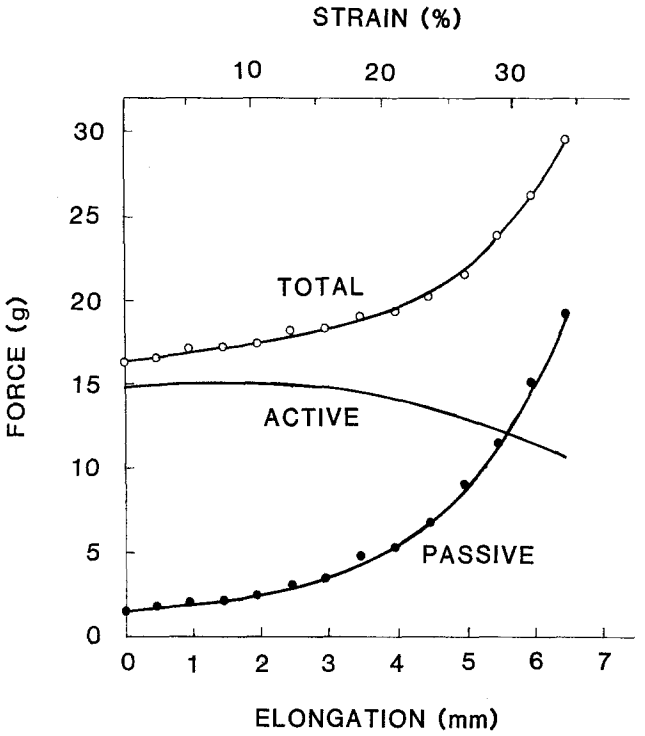

FIGURE 5. Active and passive force elongation curves. Active response shows a peak around 10% strain.

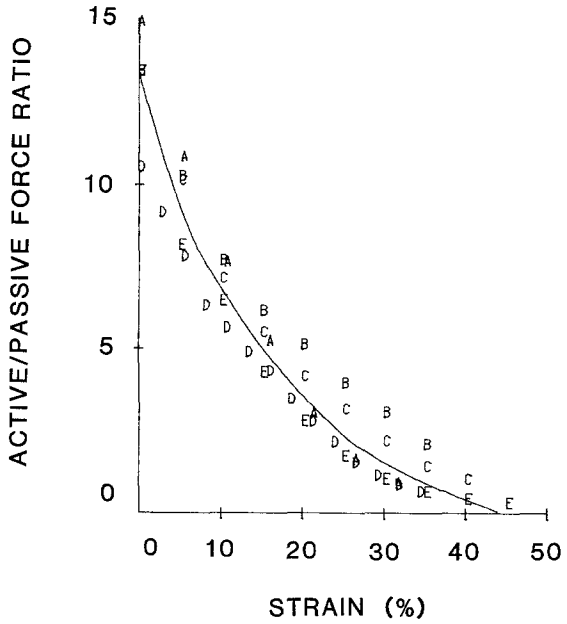

FIGURE 6. Active/passive force ratio as a function of strain. Symbols are for data points, and the solid line indicates average result.

DISCUSSION

The results of the present study confirm the findings of Hast (1966, 1967), Hirose et al. (1969), and Marteneson and Skogland (1964) that the vocalis is a fast muscle and seems to be capable of performing quick tension and length changes that may result in sudden changes in vocal pitch.

Our finding of a 22- to 32-ms contraction time for the vocalis muscle is much closer to the results of a study by Hirose et al. (1969) using cats than it is to previous results on dogs. Significant differences could be attributed to the use of different breeds of animals in the various experiments. Considerable variability in our data was associated with differences in age and size of dogs. Material from smaller dogs had briefer response times.

Additional variability in investigations of laryngeal mechanical properties could be attributed to the difference in isometric and isotonic conditions of a muscle. As indicated by Carlson and Wilkie (1974), the isotonic contraction time is shorter than the isometric value and, depending on the size of the isotonic force, could vary between zero and the isometric value. This variability may be more noticeable when an indirect approach of measuring these mechanical properties is used, as in Larson and Kempster (1985), where contraction time was inferred from changes in fundamental frequency.

Methodologically, this study demonstrated that contraction times and 50% relaxation times can be measured in vitro with specific control over elongation of the muscle.

Contrary to one of our hypotheses, twitch response time was not dependent on the length of the muscle. Over 50% strain, no noticeable trend was observed. Relaxation time, however, increased slightly with increasing length.

The maximum-active to passive force ratio for mechanical twitch was, as expected, a strong function of vocal fold length. It decreased from 13 at zero strain to less than 1 at 40% strain, indicating that passive tension in the connective tissue of the muscle assumes primary importance at larger strains.

In future studies, variability in the data could be controlled by selection of particular breeds, ages, and sex of the animals (Perlman, 1985). A word of caution must be given, however, about the general applicability of the results at this stage to the dynamics of pitch control. Although the in vitro technique for muscle stimulation is a generally accepted technique for investigations on skeletal muscle, the motor unit recruitment process is obviously eliminated. As Rack and Westbury (1969) point out, submaximal contraction properties are not merely scaled-down versions of maximally stimulated properties. Keeping this in mind, we are planning future studies to corroborate these results by measuring more natural activation patterns under brainstem stimulation. If our results are corroborated, the in vitro technique will continue to offer advantages of experimental simplicity, cost, and availability of tissue.

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