ACOUSTIC SIGNALS FROM FROG SKELETAL MUSCLE

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ABSTRACT Acoustic, force, and compound muscle action-potential signals were recorded simultaneously during maximal isometric twitches of frog gastrocnemius muscles. The onset of sound production occurred after the onset of muscle depolarization but before the onset of external force production. Acoustic waveforms consisted of oscillations that initially increased in amplitude, followed by decaying oscillations. The peak-to-peak acoustic amplitude increased with increasing temperature with a Q_{10} of 2.6 ± 0.2 over a range of 7.0–25.0°C. The acoustic amplitude increased with increasing muscle length up to ~90% of the optimal length for force generation. As length was increased further, the acoustic amplitude decreased. Microphones positioned on opposite sides of the muscle recorded acoustic signals that were 180° out of phase. These results provided evidence that sound production is produced by lateral oscillations of muscle. The oscillation frequency may provide a measure of mechanical properties of muscle.

INTRODUCTION

Contracting skeletal muscles emit continuous, low frequency sounds (Oster and Jaffe, 1980; Barry et al., 1985). A muscular etiology for these sounds was first suggested by Wollaston (1810), who heard a low rumble when he plugged his ear with his thumb and contracted the muscles of his hand and arm. The low rumble quieted when the hand and arm were relaxed. The modern investigations of human muscle sounds were initiated by Oster and Jaffe (1980) who performed experiments that demonstrated the sounds were from muscular activity and not due to blood flow or artifacts such as tremor or the microphone scraping on the skin. Barry et al. (1985) found that human muscle sounds were intrinsically tied to contraction and that sound amplitude declined during muscle fatigue while the surface electromyographic signals did not decline. Thus, the ratio of acoustic amplitude to electromyographic amplitude can be used as a measure of the loss of electromechanical coupling that accompanies muscle fatigue. Barry et al. (1986) reported the use of surface acoustic signals as the control signal for an upper extremity-powered prosthesis.

Discrete acoustic signals corresponding to individual motor units have been reported from recordings of human muscle (Gordon and Holbourn, 1948; Barry et al., 1985). Discrete acoustic signals have been recorded from frog muscle in vitro (Brozovich and Pollack, 1983, 1985), but the signal duration was too short to be generated by a motor unit. The signals recorded by Brozovich and Pollack may have been due to sliding steps between different muscle fibers (F. V. Brozovich, personal communication, 1985) and they are not related to the low frequency sounds reported here and by Gordon and Holbourn (1948), Oster and Jaffe (1980), Barry et al. (1985), or Barry et al. (1986).

Although clinical uses for muscle sounds are emerging, the mechanism of sound production remains unknown. Gordon and Holbourn (1948) hypothesized that sounds were produced by the thickening of muscle fibers during contraction. The sounds have also been attributed to some aspect of cross-bridge turnover (Oster and Jaffe, 1980). Neither of these hypotheses has previously been tested.

METHODS

Frog (*Rana pipiens*) gastrocnemius muscles were isolated and removed with the nerve supply intact from cold anesthetized animals. The tendons were sutured to steel hooks with tungsten wire or monofilament suture. The muscles were suspended between a fixed post and a force transducer (model F5A-1; Konisberg Instruments, Inc., Pasadena, CA), and placed in a bath of frog Ringer's solution (95 mM NaCl, 2.5 mM KCl, 1.8 mM Na_2HPO_4, 1.0 mM $MgSO_4$, 1.8 mM $CaCl_2$, 25.0 mM $NaHCO_3$, 5.0 mM glucose, bubbled with 95% O_2 and 5% CO_2 (Ter Keurs et al., 1978). Bath temperature adjacent to the muscle was monitored with a telethermometer (model 651; Omega Engineering, Inc., Stamford, CT). Acoustic signals were recorded by placing a hydrophone (model 8103; Bruel & Kjaer Instruments, Inc., Marlborough, MA) in the bath beside the muscle. In experiments with a single hydrophone, the hydrophone was placed ~5 mm from the muscle, with the long axis of the hydrophone perpendicular to the long axis of the muscle. In experiments with two hydrophones, the hydrophones were ~10 mm from the muscle with the long axis parallel to the long axis of the muscle.

The two hydrophones used were individually calibrated by Bruel & Kjaer Instruments Inc., and found to have a frequency response that was flat to ±1dB over a range of 1.0 Hz–30 kHz and flat to ±3 dB over 0.1 Hz–150 kHz. The hydrophone signal was amplified with a charge amplifier (model 2635; Bruel & Kjaer Instruments, Inc.); initially, filter settings were set at 2 Hz low-frequency cutoff and 100 kHz high frequency-cutoff. No signals contained power in the 1.0 kHz –100 kHz range so subsequent experiments were performed with a 1.0 kHz high-frequency cutoff. Muscle action potentials were recorded with an extracellular electrode placed near the surface of the muscle. The muscle was stimulated with a stimulator (model S88; Grass Instrument Co., Quincy, MA) and a stimulus isolation unit (model SIU-5; Grass Instrument Co.) via a suction electrode attached to the stump of the severed sciatic nerve.

Stimulation voltage was increased slightly above the level at which maximal twitches were obtained. Optimal length (L_o) was defined as the length at which the largest force was obtained. Data were recorded digitally using 12-bit analog-to-digital conversion with 0.4-ms sampling of force and acoustic signals and 0.2-ms sampling of the compound muscle action potential.

Simultaneous measurements of force development, acoustic signals, and compound muscle action potentials were recorded during maximal isometric twitches over a temperature range of 7.0°–25.0°C, for lengths from 75% to 110% of optimal length. The addition of acoustically absorbent foam on the bath walls and changes in bath chamber volume produced no variation in the acoustic signal. Muscle contraction produced small surface waves that interfered with the primary acoustic signal when the preparation or the microphone was near the bath surface. Consequently, all experiments were performed with the muscle at least 4 cm below the surface.

RESULTS

Fig. 1 shows the force, acoustic, and compound muscle action potential signals recorded simultaneously during a maximal isometric twitch. At any given temperature and length of muscle, all three waveforms were highly reproducible during repeated stimulations. The initial deflection of the acoustic waveform occurred after the initial deflection of force production. Throughout the range of temperatures and muscle lengths tested, each contraction, whether twitch or tetanic, maintained a temporal sequence of the action potential, the acoustic signal, and the force signal.

A characteristic of the acoustic waveform was an initial half-cycle smaller than the maximal half-cycle (Fig. 1). After the maximum half-cycle, the acoustic waveform displayed gradually decaying oscillations. Waveforms with as few as 2 and as many as 12 half-cycles were seen. The number of half-cycles was affected by muscle length, with longer lengths corresponding to more half-cycles.

Over the range of temperatures and muscle lengths tested, twitches and fully fused tetanic contractions (100 Hz) resulted in a single discrete acoustic signal, whereas a discrete acoustic signal was associated with each stimulus during a partially fused tetanic contraction (5 Hz). At any given length and temperature, for both twitch and tetanic contractions, the peak-to-peak amplitude of the acoustic signal was monotonically and nonlinearly related to the peak force developed. Partially fused tetanic contractions produced an initial acoustic signal with an amplitude related to the magnitude of the initial peak force. Subsequent discrete acoustic signals had amplitudes that were related to the amplitude of the ripple.

The times of the initial deflection of acoustic and force signals measured relative to the initial deflection of compound muscle action potentials during maximal isometric twitches were functions of temperature (Fig. 2). The initial deflection of the acoustic waveform appeared before the initial deflection in isometric force, but this does not prove that the acoustic signal occurred before force generation, since the initial deflection in isometric force was seen only after slack in the muscle was eliminated. However, at 25.0° ± 0.2°C the first deflection of the acoustic signal was

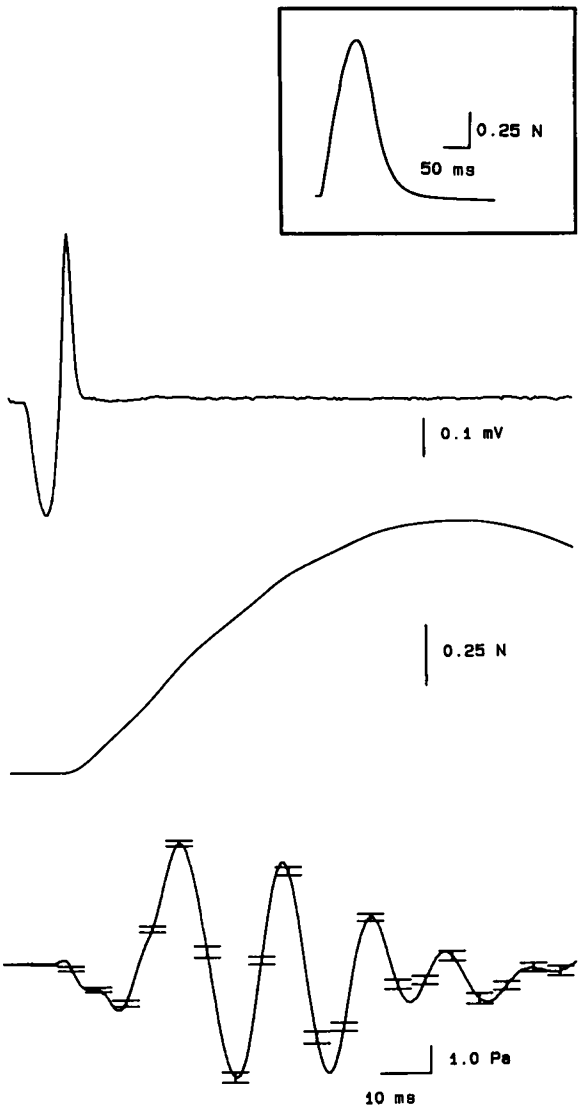

FIGURE 1 Compound muscle action potential (*top*), force (*middle*), and acoustic (*bottom*) signals were recorded simultaneously during a maximal isometric contraction at 14.0 ± 0.2°C and 90% of optimal length. The signals from 10 consecutive stimulations were averaged to produce the waveforms displayed. Error bars representing standard deviations were plotted for every 12th point of the acoustic waveform. *Inset*, the force trace on a different time scale.

2.0 ± 0.3 ms after the start of the muscle action potential, and the acoustic signal preceded force in stretched muscles where there was little slack to be eliminated. At 7.0° ± 0.2°C the entire acoustic signal was completed by the time force had risen to 50% of peak twitch force, while at 25.0° ± 0.2°C the acoustic signal was still present after tension had peaked and was decreasing.

The peak-to-peak amplitude of the acoustic signal as a function of temperature and length is plotted in Fig. 3 *A*. A Q_{10} of 2.6 ± 0.2 was obtained using least mean squared linear regression of the amplitude to a log scale of temperature. At a given temperature, the peak-to-peak amplitude of the acoustic signal increased with increasing muscle length up to ~90% of optimal length and then decreased

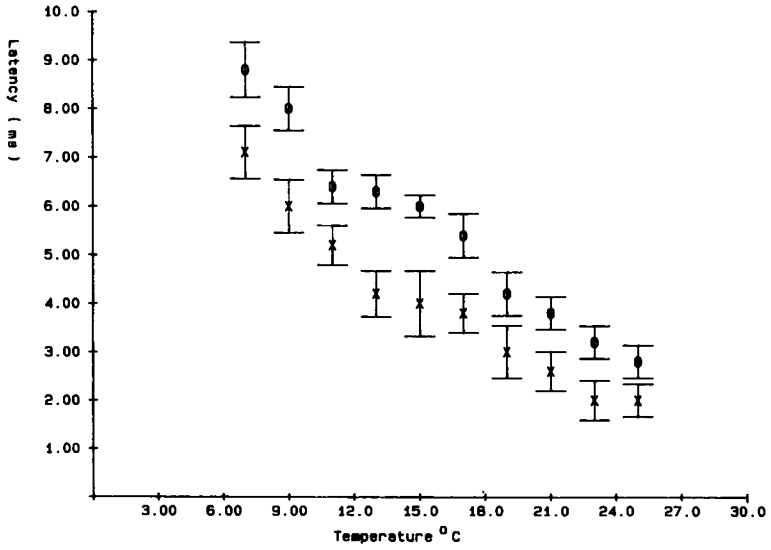

FIGURE 2 The onset of acoustic signals (×) and force production (o) was determined as a function of temperature. The onset of force production was defined as the time after the onset of the compound muscle action potential that the first point above baseline occurred. Using this definition, the force onset is at the end of latency relaxation. The acoustic signal onset was also measured relative to the onset of the compound muscle action potential. These data are average values from five experiments; the error bars denote standard deviations.

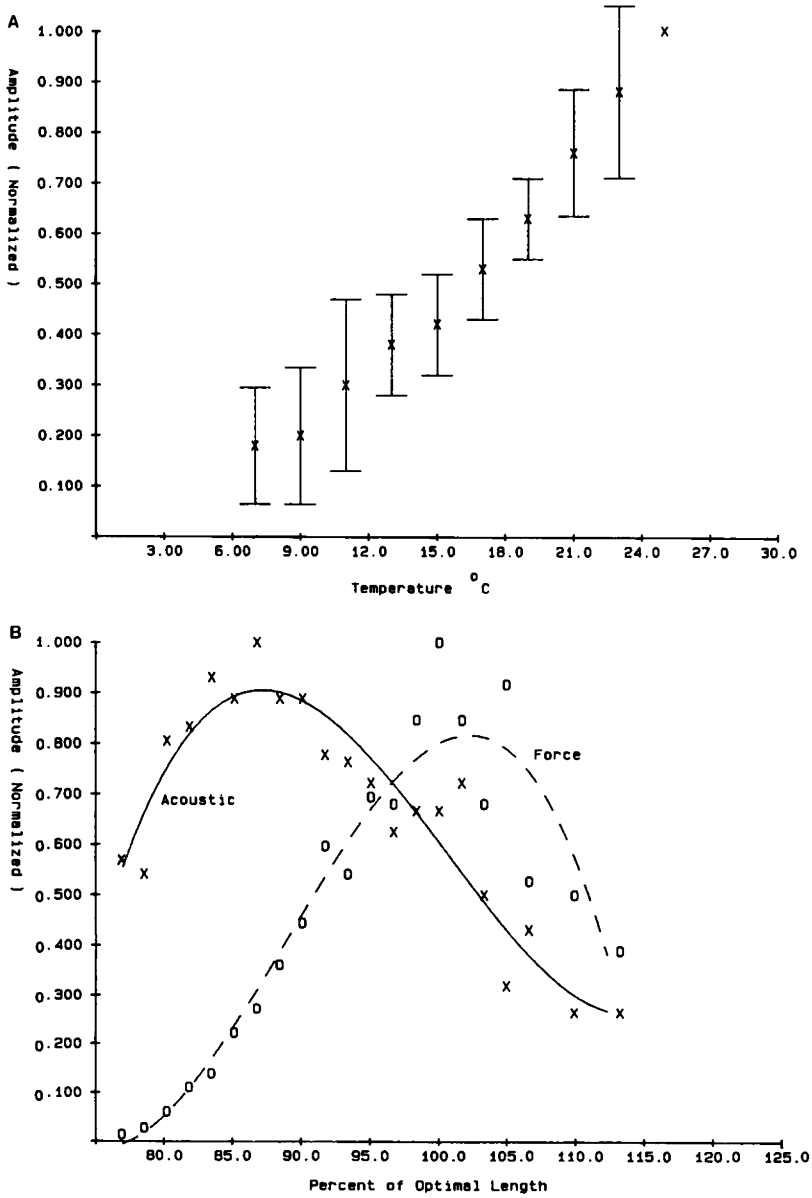

FIGURE 3 (*A*) Peak-to-peak acoustic amplitude as a function of temperature was determined at a length of 90% L_o. These data are average values from six experiments; error bars denote standard deviations. The amplitudes from each experiment were normalized to the amplitude recorded at 25.0 ± 0.2°C. (*B*) Peak-to-peak acoustic amplitude and active peak twitch force are plotted as functions of length. These data are average values from eight experiments; the amplitudes from each experiment were normalized to the maximum ampitude recorded. Muscles became slack below ~80% of the optimal length for force production, but the muscles still contracted and emitted sounds. *Solid and dashed curves*, the third order least mean squared polynomials fit to acoustic and force data, respectively.

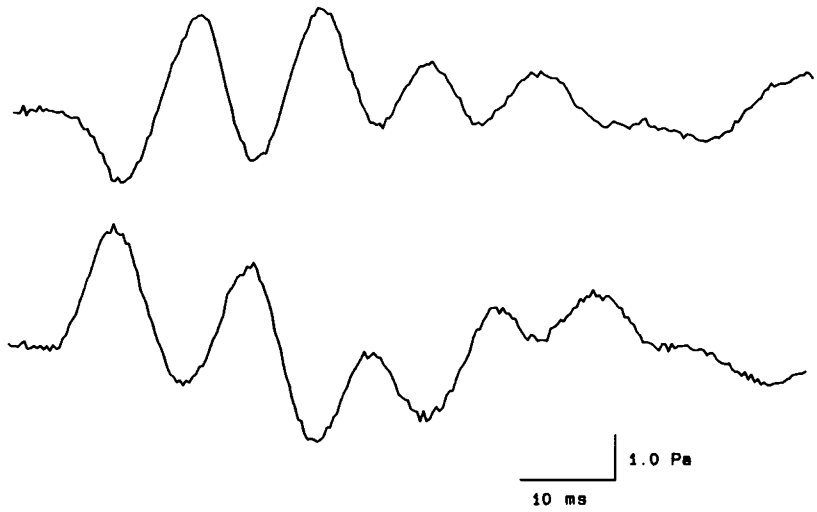

FIGURE 4 Acoustic signals recorded simultaneously by two hydrophones placed on opposite sides of a muscle revealed a phase relationship of 180°.

(Fig. 3 *B*). The curve for the relationship between the acoustic signal and length was similar to that of the length-force curve, but the optimal length for the amplitude of the acoustic signal occurred at a shorter length than that for force development. At lengths shorter than ~80% of optimal length, muscles were slack. At these lengths, no external force was measured, but sounds were still produced while muscles were observed to contract.

Fig. 4 shows acoustic signals recorded simultaneously by two hydrophones placed on opposite sides of the muscle. The signals were 180° out of phase and, with the hydrophones kept 180° apart, the phase relationship of the acoustic signals remained at 180° as the hydrophones were rotated around the long axis of the muscle.

DISCUSSION

An acoustic waveform produced by a muscle twitch is characterized by oscillations that initially increase in amplitude and then decrease. These oscillations are consistent with an etiology of muscle movement perpendicular to the long axis of the muscle producing muscle sounds. Muscle fiber thickening as an etiology is consistent with the effects of changing temperature and muscle length on sound production, but thickening alone should produce sounds that are radially symmetric with respect to the long axis of the muscle. The result that sounds from opposite sides of the muscle are 180° out of phase is inconsistent with thickening as an etiology. Lateral movement of the muscle is required to produce the phase relationship measured. Lateral movement of the muscle can occur as slack in the muscle is being reduced so acoustic signals can appear by this mechanism prior to measured force production. During isometric contractions, lateral movement is limited by the internal compliance of the muscle and is reduced by stretching the muscle. The reduction of muscle sound amplitude with muscle lengths >90% of L_0 is consistent with lateral muscle movement as the etiology of sound production. The reduction in amplitude at lengths shorter than 90% L_0 remains unexplained. One possibility is that, as the muscle becomes slack, the geometry of the muscle–microphone system changes and sound energy is simply not transmitted as efficiently. The absence of sounds during the plateau of a fused tetanic contraction is also consistent with the lateral movement hypothesis since no significant lateral movements can occur during the plateau phase.

The lateral oscillations should occur at a frequency corresponding to the resonant frequency of the muscle. The resonant frequency of a material is determined by the geometry, mass, viscosity, and stiffness of the material. As force increases, the longitudinal stiffness increases in muscles and muscle fibers because of increasing numbers of attached cross-bridges (Ford et al., 1981). Since muscle is not a homogeneous material, longitudinal and transverse mechanical properties may differ significantly. Nevertheless, transverse oscillations should exhibit a resonant frequency related to longitudinal stiffness. Qualitatively, the acoustic signal increases in frequency as force increases (Fig. 1). Quantitative measures of the relationship between acoustic frequencies and muscle stiffness have not yet been made.

The period of the first half-cycle of oscillation should be related to the rate of the initial phase of contraction. The combination of structural information from the later phases of oscillation and contraction rate information from the initial phase may allow acoustic signals to be used to measure contractile properties from individual muscles in vivo.

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