Alteration of Tension Response to Stretch with Spontaneous Contraction Frequency in the Guinea Pig Taenia Coli and Stiffness – Work Characteristics

N. OZTURK and P. UNGAN

Hacettepe University, Faculty of Medicine, Department of Biophysics, Ankara, Turkey

Abstract. Previously we developed a data acquisition procedure to measure the tension responses of smooth muscles during their spontaneous contraction. This study was undertaken to determine whether the time course of the tension responses to stretches was altered with the ongoing spontaneous contractions. In addition, the stiffness, phase and work characteristics were evaluated from the tension responses in order to see how these characteristics were affected by the changes in the tension responses. The tension responses of the spontaneously contracting taenia coli to stretches had four phases. However, the latencies of the phases changed with the type of the spontaneous contractions during which the measurement was carried out. In the responses recorded during the slow spontaneous contractions (40–70 s), the second phase reached the minimum at 2.26 ± 1.12 s and the third phase had the peak tension at 9.56 ± 3.08 s. These latencies were 0.89 ± 0.30 s and 3.91 ± 1.10 s, respectively, when the experiments were undertaken during high frequency contractions (10–30 s). Besides, the maxima and minima in the stiffness, phase and work characteristics were also shifted to higher frequencies as the spontaneous contraction became faster. Work had a positive peak around 0.03 Hz for the slowly contracting taenia coli. The peak shifted to 0.1 Hz when the taenia coli contracted at higher frequency. The results show that the time course of the tension responses is determined by the contractile state of the taenia coli.

Key words: Taenia coli — Tension response — Stretch — Stiffness – Work characteristics

Introduction

When tetanically contracting skeletal muscle is subjected to a sudden length chan-

Correspondence to: Dr. Necla Ozturk, Hacettepe University, Faculty of Medicine, Department of Biophysics, 06100 Ankara, Turkey

ge, the tension response displays a characteristic feature with four phases. Moreover, it is proposed that these phases are associated with some steps within the cross bridge cycle. Specifically, it is believed that the early recovery phase of the tension responses corresponds to the transition rate from a lower to a higher force producing state while the third phase (the delayed tension reversal) is considered to be related to the dissociation of heads in high force producing state (Huxley and Simmons 1971; Ford et al. 1977). The tension responses have been measured in smooth muscle too, as a means to probe the contraction mechanisms related to the sudden length change (Hellstrand and Johansson 1979; Warshaw and Fay 1983a,b; Peiper et al. 1984; Yamakawa et al. 1990; Arheden and Hellstrand 1991). However, studies on smooth muscle have not revealed tension responses similar to those in skeletal muscle. The responses recorded during steady contraction exhibit an abrupt change in the tension in the same direction as the length change. Thereafter, the tension decays (or rises) and reaches steady state. In addition, in these studies, experiments were carried out at low temperatures in order to suppress spontaneous contractions; and therefore the results reflect the mechanics of smooth muscle at low temperature. Recently we have developed a data acquisition procedure to record the tension responses of smooth muscle to sudden length changes during their rhythmic spontaneous contractions (Öztürk 1993). Besides, we could determine four phases in the tension responses, and we proposed that the four phases were related to the properties of the contractile system activated by stretch.

The present study was undertaken in order to show explicitly that the contractile state of smooth muscle was reflected to the time course of the tension responses. In order to achieve the goal, the tension responses of the taenia coli were recorded during the development of two different rhythmic contraction patterns; namely, during the generation of slow contractions with a period of 40–70 s, and fast contractions with a period of 10–30 s. Besides, stiffness and work characteristics were also evaluated to see how these characteristics were altered with the contractile state of the muscle.

Materials and Methods

Female guinea-pigs with a body weight of 400–500 g were used for the present experiments. The animals were killed by a blow to the neck. Strips of taenia coli (about 15 mm in length) were dissected out. After the isolation, the preparations were mounted vertically in a 50 ml organ bath containing Krebs solution at 36 °C. The composition of the Krebs solution was (in mmol/l): NaCl 118, KCl 4.7; $CaCl_2$ 2.5, $NaHCO_3$ 14.9, KH_2PO_4 1.18, $MgSO_4$ 1.17, glucose 5.5. The upper end of the muscle strip was attached to a Grass FT. 03 force-displacement transducer and the lower end was connected to the lever of a linear motion displacement transducer. The force transducer was mounted in a specially designed micrometer so that the preparation could be adjusted to the desired length. In order to impose length changes to the muscle strip, the lever of the displacement transducer was attached to the core of an electromagnetic vibrator. The overall compliance of the mechanical system was 2 mm/N and the frequency response of the mechanical system was flat within the frequency range of 0–20 Hz. The length and tension signals from transducers were first amplified and then converted to digital signals by means of an eight channel, 12 bit A/D converter and stored on a computer for off-line analysis.

After mounting, the muscle strips were allowed to equilibrate under a passive force of about 5 mN for 1 hour. Then the isometric spontaneous contractions of the taenia coli were recorded. While rhythmic spontaneous contractions with either a period of 10–30 s or 40–70 s were being recorded, consecutive sudden stretches and releases were imposed continuously on the spontaneously contracting taenia coli. The amplitude of the length perturbations was maintained at about 1% of the muscle length and the rise time of the length change was 10 ms. The duration of the stretch/release was set to 35 s when the spontaneous contractions were fast (10–20 s) and to 65 s when the spontaneous contractions were slow (40–70 s). Tension and length signals were sampled with a frequency of 100 Hz for the stimulus duration of 35 s, and 50 Hz for the stimulus duration of 65 s. The recording was started in the instant when the length signal was altered from release to stretch direction. A record length (epoch) was adjusted such that it could cover the whole duration of the length perturbation in the direction of stretch. Sampling of the tension and length signals was carried out simultaneously. However, the tension records taken during the continuous application of the length perturbations to isometrically contracting muscle were actually the superimposition of the low-frequency ongoing spontaneous contraction and the tension response to the sudden length perturbation. So, to eliminate the spontaneous contractions and to elicit the tension response to stretches, tension records (epochs) belonging to each successive stretch were added on-line onto one another. Hence the average of the successive epochs was evaluated. Since the application of the consecutive stretch and release length perturbations occurred randomly in relation to the time course of the spontaneous contraction, the amplitude of the spontaneous contractions in the average record was increased by a factor of the square root of the average number, while the amplitude of the phase locked tension responses increased in proportion to the average number. As a result, the ratio of the amplitude of the tension response to the amplitude of the spontaneous contractions increased as the averaging process continued. The averaging process lasted until an invariant time course of a tension response to stretch was obtained. Simultaneously with the tension, the average of the length perturbations was also carried out (Öztürk 1993).

After the experiments, the tension responses were analyzed in time and frequency domains. For the analysis in the time domain, the latencies of the components of the tension responses were measured. Analysis of the responses in the frequency domain was carried out using the FFT algorithm. During the experiments, the sampling interval was adjusted either to 10 or 20 ms in order to have a better time resolution. However, when the data were subjected to Fourier transform, they were rearranged by taking every third or sixth point. So the sampling interval for the calculation was increased to 60 ms. This arrangement of the data provided a frequency resolution of 0.016 Hz with a data number 1024 and an upper frequency of 4 Hz (on the basis of four points per cycle). Initially, Fourier transforms of the average tension response to stretch and length signal were evaluated. Thereafter, the ratio of the modulus of the tension response to the modulus of the length was calculated in order to find the stiffness for different frequencies of the length perturbation. Then the stiffness values were converted to the values in dB and were normalized by subtracting the value at 0 Hz. Phase values ($\Phi(\omega)$) were obtained by taking the difference of the phase angles between the tension and length signals for each frequency. Positive phase means that tension leads the length changes. The variations of the stiffness and phase angles with the frequency were plotted on a semilogarithmic paper and illustrated in the form of Bode diagrams. The mechanical work done for each cycle was calculated using the equation

$$W(\omega) = -2\pi \cdot |F(j\omega)| \cdot |L(j\omega)| \cdot \sin\Phi(\omega)$$

Each value was divided by the maximum work and thus they were expressed as normalized values. Also the variation of work with the frequency was plotted on a semilogarithmic scale.

Data subject to statistical variation are reported as means $\pm$ standard error with the number of observations (n) in parentheses. Test of significance was performed with Student's t test with pooled data analysis.

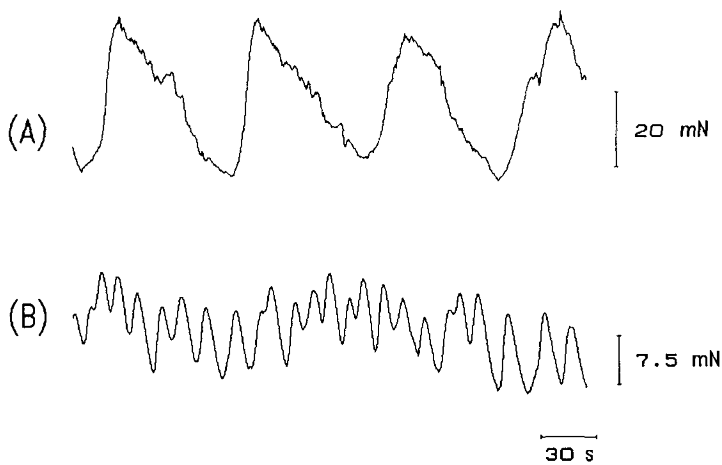

Figure 1. Two typical spontaneous contraction patterns recorded from the guinea pig taenia coli. These contraction patterns are typical in the sense that the one at the top (A) is an example for the slow contractions and the one at the bottom (B) is for the high frequency contractions. Each pattern was recorded from a different strip.

Results

In order to determine whether the contractile state of the taenia coli is reflected to the time course of the tension responses to stretch, experiments were carried out during the development of spontaneous contractions at two different frequency levels explicitly, during slow contractions with a period of 40–70 s, or fast contractions with a period of 10–30 s. For this purpose 13 experiments were undertaken, each on a different taenia coli strips. Of these, 7 had the slow spontaneous contractions and their contraction patterns were similar to the one in Fig. 1*A*. On the other hand 5 of the muscles had the high frequency spontaneous contractions similar to the pattern in Fig. 1*B*. The tension responses recorded from 13 strips depicted a common feature consisting of four phases. The first phase appeared as a sudden increase in tension. Then the tension decreased and reached a minimum (the second phase). Afterwards the tension increased again (the third phase) and led to steady state (the fourth phase). However, these responses differed in their time basis, depending on the type of the spontaneous contractions going on while the tension responses were recorded. The second and the third (tension rise) phases were elicited at a later time when the spontaneous contractions were slow, and at an earlier time when the spontaneous contraction were fast. In the responses recorded during the slow spontaneous contraction, the second phase reached its minimum at 2.26 ± 1.12 s and the third phase had the highest tension at 9.56 ± 3.08 s ($n = 7$). On the other hand, in the responses measured during the high frequency spontaneous contractions, the latencies for these two phases were 0.89 ± 0.30 s and 3.91 ± 1.10 s ($n = 5$), respectively. When the latencies of the second and the third phase in the two types of responses were compared, it was found that they were different significantly ($p < 0.05$). When the taenia coli strips were left in a medium containing papaverine, the time locked third phase disappeared completely; and the responses were similar to those of viscoelastic materials.

Typical tension responses of the taenia coli to sudden stretches as measured during the slow (the response at the top) and the high frequency (the response at the bottom) spontaneous contractions are shown in Fig. 2. In this figure, the shift in the latencies are also clearly seen.

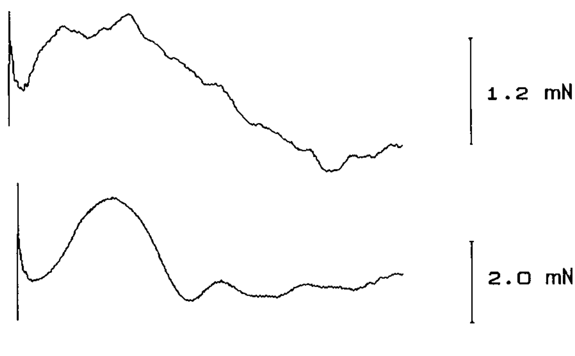

Figure 2. Tension responses of the guinea pig taenia coli to stretch, recorded during the development of the slow type of spontaneous contractions (top), and during the high frequency contractions (bottom). These responses were obtained by on-line averaging of 60 and 125 single records, respectively. The time scale is 10 s for the first, 5 s for the second response.

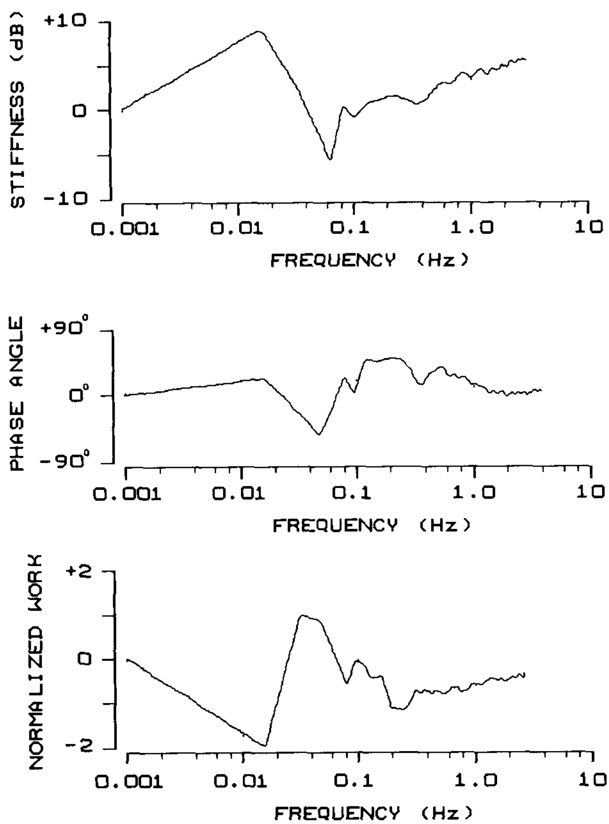

Figure 3. Stiffness, phase and work characteristics evaluated from the responses measured during the slow contractions. These characteristics are the average of the results obtained from seven teania coli strips.

The variation of the time course of the tension responses with the ongoing spontaneous contractions was also reflected to the stiffness and phase characteristics. The stiffness characteristics evaluated from the responses of the slow type displayed a maximum and a minimum feature. The stiffness had high values at lower frequencies. As the frequency changed from 0.01 to 0.08 Hz, the stiffnes decreased, and it had a minimum around 0.06–0.08 Hz. Then the stiffness increased again as the frequency was elevated. The phase angle between the tension response and the length perturbation also demonstrated a marked frequency dependency. The phase shift was slightly positive around 0.01–0.02 Hz. Between 0.02–0.07 Hz, tension response lagged behind the length perturbation. Within this frequency range, the maximum lag was observed around 0.04–0.05 Hz and its value was about 50°. However, at about 0.06 Hz, phase response changed its direction and,

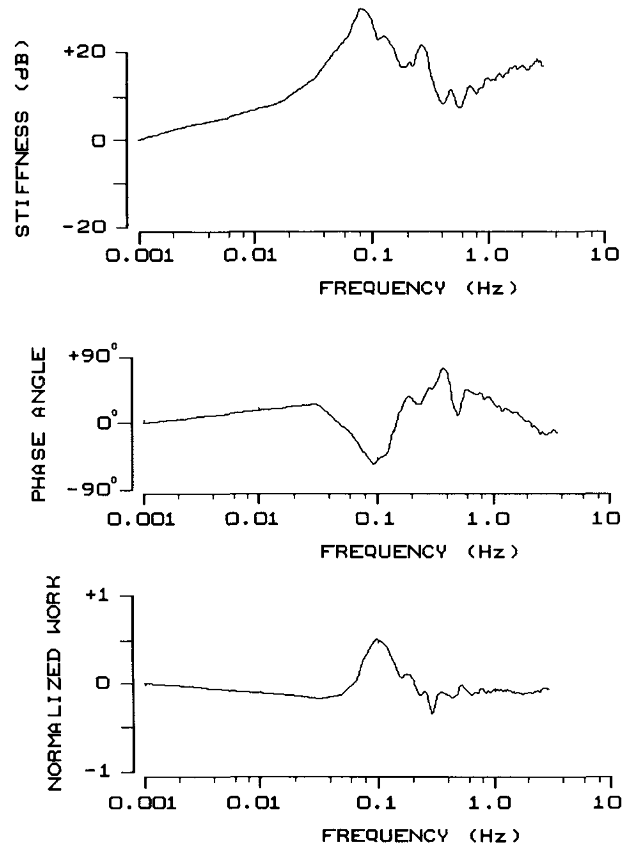

Figure 4. Stiffness, phase and work characteristics evaluated from the responses measured during the high frequency contractions. These characteristics are the average of the results obtained from five teania coli strips.

for the frequencies over 0.08 Hz, the tension response led the length change. The mechanical work characteristics had positive values within the frequency range of 0.02–0.06 Hz and they reached the peak around 0.03 Hz. Fig. 3 shows the stiffness (top), phase (middle) and work (bottom) characteristics evaluated from the tension responses of the slow type. They are the average of the characteristics obtained from seven taenia coli strips.

The stiffness, phase and work characteristics obtained for the taenia coli strips with high frequency spontaneous contractions, in general, were similar to those evaluated for the muscles with slow contractions, except that the stiffness characteristics had never a minimum. In addition, the frequency region where the phase angles had negative values was shifted to higher frequencies. Also the positive peak in the work characteristics was shifted to higher frequencies. The Bode diagrams in Fig. 4 show this type of characteristics. They are the average of the characteristics evaluated from five tension responses which were recorded during high frequency spontaneous contractions. In these characteristics, the maximum negative phase shift occurred around 0.1 Hz and its value was about 55°. Besides, work reached its peak at 0.1 Hz.

Discussion

The important result of this study was that the latencies of the components in the tension responses altered with the frequency of the rhythms in the spontaneous contraction patterns during which the tension responses were measured. Specifically, the second and the third phase delayed as the spontaneous contractions became slower. Moreover, the third phase disappeared completely and the time course of the second phase changed when papaverine was added to the bathing medium. Therefore, we can deduce that the phases in the tension responses are genuine responses. In addition, these results also implicate that the time course of tension responses are correlated with the state of contractile elements during which the length perturbations were being applied. Besides, the peculiar feature of the stiffness, phase and work characteristics was also altered with the type of the responses they were evaluated from. In the characteristics evaluated from the tension responses of slowly contracting taenia coli, the minimum in stiffness occurred around 0.06–0.08 Hz and positive work reached the peak at 0.03 Hz. On the contrary, in most of the characteristics evaluated from the fast type of tension responses, we could not observe a minimum in the stiffness. In addition, in this type of characteristics, the maximum positive work was observed at 0.1 Hz. These results also show that the variations in the spontaneous contraction patterns and hence the contractile state of the muscle were reflected to the stiffness, phase and work characteristics, and these characteristics also should be associated with the properties of the contractile system. Furthermore, a positive work means that the muscle strip is doing work on the measuring system. In other words, the frequency at which he positive work reaches the peak corresponds to the resonant frequency of the muscle strip. In fact, a behaviour similar to a resonance has been observed in skeletal and cardiac muscle too. The rabbit and guinea pig heart muscle had a tension-length diagram in a counterclokwise direction when they were stretched and released sinusoidally with a frequency of 0.1–0.8 Hz (Steiger 1971). Beyond this frequency range they had a clockwise tension length diagram. This indicates that, within 0.1–0.8 Hz, the work during the shortening half cycle is greater than during the stretch half cycle and the heart muscle is doing work on the mechanical apparatus (Steiger 1971). Similarly, the tension length diagrams of insect flight muscle during sinusoidal stretch and release had counterclockwise direction within the frequency region of 1–10 Hz (Steiger and Rüegg 1969). Besides, in skeletal and cardiac muscle, the variation of the stiffness and phase with the frequency of the length perturbation revealed a peculiar feature. The stiffness characteristics displayed a marked minimum (Saeki et al. 1978; Rossmanith et al. 1980; Barden 1981; Rossmanith 1986; Shibata et al. 1987). Phase responses had negative values around the frequency range where the stiffness was minimum. However, at the minimum, phase values changed abruptly from negative to positive values. Within the region where the phase angle was negative, the work done per cycle had positive values. Furthermore, it was found that the ATPase activity was the highest at the frequencies where the positive work was observed (Steiger and Rüegg 1969). Therefore the frequency at which the muscle strip is doing positive work is assumed to be related to the cross bridge cycling rate. Since there is some divergence between the mechanics of smooth muscle and skeletal or cardiac muscle, it may lead to a misleading conclusion to put a direct correlation between the resonant frequency (positive work region) determined in this study and the cycling rate of the cross bridges that are involved in the generation of the spontaneous contractions in the taenia coli. However, at least we can propose that stretch activated contraction mechanism in the taenia coli has a component about 0.03 Hz when it contracts slowly and 0.1 Hz when it contracts faster. On the basis of these findings, we can claim that the method implemented here can be used to assess the responses of smooth muscles to sudden stretch.

References

Arheden H., Hellstrand P. (1991): Force response to rapid length change during contraction and rigor in skinned smooth muscle of guinea-pig taenia coli. J. Physiol. (London) **442,** 601—630

Barden J. A. (1981): Estimate of rate constants of muscle cross bridge turnover based on dynamic mechanical measurements. Physiol. Chem. Phys. **13,** 211—219

Ford L. E., Huxley A. F., Simmons R. M. (1977): Tension response to sudden length change in stimulated frog muscle fibers near slack length. J. Physiol. (London) **269,** 441—515

Hellstrand P., Johansson B. (1979): Analysis of the length response to a force step in smooth muscle from rabbit urinary bladder. Acta Physiol. Scand. **106,** 221—238

Huxley A. F., Simmons R. M. (1971): Proposed mechanism for force generation in striated muscle. Nature **233,** 533—538

Öztürk N. (1993): An approach for measurement of tension transient responses of smooth muscles during their spontaneous contractions. Acta Physiol. Scand. **148,** 227—228

Peiper U., Vahl C. F., Donker E. (1984): The time course of changes in contraction kinetics during the tonic activation of the rat tracheal smooth muscle. Pflügers Arch. **402,** 83—87

Rossmanith G. H. (1986): Tension responses of muscle to n-step pseudo-random length reversals: a frequency domain representation. J. Muscle Res. Cell Motil. **7,** 299—306

Rossmanith G. H., Unsworth J., Bell, R. D. (1980): Frequency-domain study of the mechanical response of living striated muscle. Experientia **36,** 51—53

Saeki Y., Sagawa K., Suga H. (1978): Dynamic stiffness of cat heart muscle in Ba^{2+}-induced contracture. Circ. Res. **42,** 324—333

Shibata T., Hunter W. C., Yang A., Sagawa S. (1987): Dynamic stiffness measured in central segment of excised rabbit papillary muscles during barium contracture. Circ. Res. **60,** 756—769

Steiger G. J. (1971): Stretch activation and myogenic oscillation of isolated contractile structures of heart muscle. Pflügers Arch. **330,** 347—361

Steiger G. J., Rüegg J. C. (1969): Energetics and efficiency in the isolated contractile machinery of an insect fibrillar muscle at various frequencies of oscillation. Pflugers Arch **307,** 1—21

Warshaw D. M., Fay F. S. (1983a): Tension transients in single isolated smooth muscle cells. Science **219,** 1438—1441

Warshaw D. M., Fay F. S. (1983b): Cross-bridge elasticity in single smooth muscle cells. J. Gen. Physiol. **82,** 157—199

Yamakawa M., Harris D. E., Fay F. S., Warshaw D. M. (1990): Mechanical transients of single toad stomach smooth muscle cells. Effects of lowering temperature and extracellular calcium. J. Gen. Physiol. **95,** 697—715

Final version accepted March 30, 1994