Effect of Duration of Stimulation on Mechanical Properties of Trachealis Muscle

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SASAKI, H., HOPPIN, F.G., Jr. and TAKISHIMA, T. Effect of Duration of Stimulation on Mechanical Properties of Trachealis Muscle. Tohoku J. exp. Med., 1986, 150 (3), 259-271 —— We have studied the effect of duration of stimulation on the dynamic behavior of isolated dog trachealis muscle tonically contracted by alternating current or carbachol. With the muscle contracted quick stretch and quick release maneuvers were performed. The force-length curves during the quick maneuvers were similar for both methods of stimulation and regardless of the duration of stimulation. This behavior is consistent with an undamped passive series elastic component. The plateaux of force preceding these quick maneuvers were also similar. Isometric stress relaxations after quick stretch were similar. However, after quick release, isometric stress recovery and isotonic shortening velocity were more rapid with alternating current stimulation than with carbachol, and both isometric stress recovery and isotonic shortening velocity decreased with increasing duration of stimulation by either means of stimulation. We conclude that the difference in velocity of shortening is not due to the different method of stimulation but rather to the duration of stimulation. Active respiratory smooth muscle may show different dynamic properties depending on the duration of stimulation but not on the differences in means of stimulation. ——— alternating current stimulation; carbachol; series elastic component; stress recovery; shortening velocity

In a previous study (Sasaki and Hoppin 1979) on excised strips of trachealis muscle stimulated either with alternating current (AC) or carbachol, it was found that there were differences in behavior during length-cycling maneuvers and during isometric stimulation. There were larger force-length loops during length-cycling of the AC-stimulated muscle than were seen at the same cycling frequency with carbachol stimulation. We had not anticipated such differences in behavior because the mechanism of stimulation is reputedly similar; atropine almost completely blocks the response to AC (Stephens and Kroeger 1980). We did however, note the major difference in duration of stimulation in our experiments;
we had used AC stimulations lasting less than 60 sec whereas the state of active constriction by carbachol had been many times greater. We then postulated that the differences observed during cycling might be related not to the nature of the stimulus (Hardung and Laszt 1966) but rather, after the suggestion of Somlyo and Somlyo (1968), to the duration of stimulation. To test this postulate, we set out to characterize the contractile properties of excised dog trachealis muscle at different durations of stimulation by both means of stimulus. In particular we have studied isometric quick-stretch and both isotonic and isometric quick-release maneuvers of stimulated muscles and analyzed the results in terms of the behavior of the series elastic component (SEC) and the contractile element (CE) (Hill 1938).

We have reported the present study previously in abstract (Hoppin and Sasaki 1978).

METHODS

Twenty two mongrel dogs were anesthetized with 30 mg/kg pentobarbital administered intravenously and were killed by overdoses of pentobarbital. The trachea, 8-10 cm in length, was removed. The end of the trachea near the carina was cut transversely between cartilages to provide one or two complete tracheal rings for study. The ring (Fig. 1 left lower) was opened ventrally and everted, causing the trachealis muscle and tunica fibrosa to separate (Stephens et al. 1969). The tunica fibrosa was then cut away and the preparation dissected so that the muscle was free except at its insertions into the cartilages. Both cartilages were trimmed to about 5 mm in length and connected to the clips in a tissue bath for force-length studies (Fig. 1 right lower). The width of each ring was about 5 mm and the thickness about 0.5 mm in the resting state.

The remaining intact excised trachea was mounted in another tissue bath. The two ends were securely tied around rubber corks and those corks were mounted at resting tracheal length. Isoproterenol 1 mg/ml and atropine 5 mg/ml were added to the bath to relax the muscle completely. The trachea was inflated to 30 cm H2O. The circumference of the inflated trachea was estimated with a string gently passed around the middle portion of the specimen. From this circumference, we subtracted the lengths of cartilage cut away. This calculation gave the length of the trachealis muscle in situ with the trachea distended by 30 cm H2O transmural pressure, L\textsubscript{sm,max}.

The two tissue baths contained Tyrode's solution with composition in mM of NaCl 136.9, KCl 3.4, NaH2PO4 0.5, MgCl\textsubscript{2} 1.3, NaHCO\textsubscript{3} 11.9, CaCl\textsubscript{2} 2.3, dextrose 5.6. Gas containing 95% O\textsubscript{2} and 5% CO\textsubscript{2} was continuously bubbled from the bottom of the bath and the system was maintained at 37°C ± 0.1. If a second trachealis strip was to be studied, the ring was maintained in Tyrode's solution bubbled with a gas mixture but at a temperature of 4°C for up to 4 hr. About 10 min elapsed between the death of the dog and the mounting of the first strip and the tracheal segment in the baths.

After mounting the muscles in the warm oxygenated Tyrode's solution, they were stretched to 85% of the L\textsubscript{sm,max}. In the previous study (Sasaki and Hoppin 1979), the peak force developed during AC stimulation was found to be at this length.

The apparatus in which the muscle strips were suspended (Fig. 1 right), based on the method of Jewell and Wilkie (1958), allowed us to measure force, F\textsubscript{sm}, and length, L\textsubscript{sm}, during stimulation by AC or carbachol, at constant length or with quick stretches or quick releases to constant length (isometric studies) or at constant force (isotonic studies). The trachealis muscle (M) was mounted vertically with the cartilage fragment at one end held by a fixed clip and at the other by a clip connected to a thin rod (R). For isometric...
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During experiments, this rod was directly connected to an isometric force transducer (F) (Series 363, Harvard Apparatus Co., Millis, MA, USA). The force transducer in turn was mounted on a vertical microscope rack and could be fixed or rapidly moved by turning the knob of the rack. The position of the transducer ($L_{sm1}$) was recorded by a linear differential transducer, and the force by F. Alternatively, for “isotonic” conditions, the rod R is attached to rod B. The length of the smooth muscle then is recorded by the linear differential transducer, $L_{sm2}$, and the load on the muscle is obtained from the sum of the appropriately calibrated forces exerted by the weight (W) and the oil-filled dashpot (D).

Fig. 1. Schematic diagrams of the experimental set up. Left lower, a ring of trachea showing, cross hatched, the portion mounted for study. Right, lower, the muscle (M) is mounted in a bath. The rod (R) can be attached to the isometric force transducer, (F), the position of which can be changed manually by rotating the knob. In this configuration the length of the muscle is recorded by the linear differential transducer, $L_{sm1}$, and the force by F. Alternatively, for “isotonic” conditions, the rod R is attached to rod B. The length of the smooth muscle then is recorded by the linear differential transducer, $L_{sm2}$, and the load on the muscle is obtained from the sum of the appropriately calibrated forces exerted by the weight (W) and the oil-filled dashpot (D).
the isotonic quick releases and provided approximately 1/4 of the afterload for the highest velocities.

The signals from Lsm1, Lsm2, and F were recorded on a tape recorder (PS 207A Precision Instrument Co., Boston, MA, USA) and an oscillograph (7712, Hewlett Packard, Waltham, MA, USA). Data from the tape recorder were traced on an oscilloscope (Tektronix 5103 N, Beaverton, OR, USA) and recorded with a polaroid camera.

Two forms of stimulation were used. Electrical field stimulation was made through 2 platinum plates suspended in the bath on either side of the trachealis preparation. The plates were 0.7 x 4.5 cm and lay 1.3 cm apart. Stimuli were at 60 Hz and had either supramaximal field strength from 5 V and 400 mA to 9 V and 700 mA. Duration of stimulus was generally between 30 to 60 sec at a 10 min interval between stimuli. Alternatively, carbachol was added to the circulating Tyrode's solution for a final concentration of $5 \times 10^{-8}$ g/ml or $10^{-5}$ g/ml.

To determine the effect of the duration of stimulation, we performed quick stretch and quick release maneuvers after 6, 10 and 30 to 60 sec of AC stimulation and at 1 or 2, 5, 15 and 30 min after addition of carbachol in 18 muscle strips from 15 dogs. The order of these intervals after AC stimulation or addition of carbachol were randomized. For quick stretch or quick release to isometric conditions, the strip was first stimulated at 85% $L_{sm}$ max. Then, after the appropriate interval, the knob was quickly turned to shorten or lengthen the strip by 2 to 10% $L_{sm}$ within 100 msec and the time-course of force was observed over the next 15 sec or more. For quick release to isometric conditions, the rod, (R), was connected to bar (B) but was initially hooked over the force bar (F) of the isometric apparatus. Stimulus was initiated with the muscle at 85% $L_{sm}$ max. The initial $F_{sm}$ was the sum of the forces recorded at F and exerted by the fixed weight, (W). At the appropriate interval, the hook was dislodged from the isometric force bar (F) and the ensuing changes of length were recorded by the record linear differential transformer, $L_{sm2}$. The isotonic loads were 19% ± 5% (s.d.) of the prerelease loads. The initial rapid shortening lasted approximately 50 msec and was less than 10% $L_{sm}$ max. The subsequent shortening was much slower and maintained a nearly constant velocity over a few sec. The extent that the velocity was constant, the load of the dashpot was constant and the conditions were isotonic.

Fig. 2. Typical tracings of muscle length, $L_{sm}$, and force, $F_{sm}$, against time in stimulated muscles. During quick stretch (or release) the $F_{sm}$ initially increases (or decreases). This initial behavior is taken to represent the behavior of the series elastic components, SEC. The behavior after completion of the quick stretch or release is interpreted in terms of the contractile element, CE. At constant length after quick stretch (left), $F_{sm}$ falls slowly (isometric stress relaxation). At constant length after quick release (middle), $F_{sm}$ rises slowly (isometric stress recovery). At constant force after quick release (right, the muscle continues to shorten at reduced velocity (isotonic shortening).
Fig. 3. Oscilloscopic tracings, redrawn from photographs taken by Polaroid camera, shows the relationship of length and force in the active trachealis muscle during several quick releases of varying amplitudes from an initial length of $85\%$ of $L_{sm\text{max}}$. The solid tracings represent quick releases performed 10 sec after the AC stimulation and the dashed tracings represent quick releases performed 30 min after the addition of carbachol to the bath. The relationship, taken to represent the properties of the SEC, appears unchanged by the duration of stimulation. Dots show the ends of varying amplitude of shortening.

Fig. 4. Rate of isometric stress relaxation after quick stretch (upper panel) and force immediately preceding stretch (lower panel) plotted against the duration of stimulation. Solid lines connect measurements made in the same strip during AC stimulation, dashed lines during carbachol stimulation. Symbols refer to individual dogs. Rates of stress relaxation were calculated as the fractional return to the pre-stretch baseline in 2 sec. They show no systematic change with duration of stimulation.
To consider the effect of time-path-dependency, we obtained quick releases from different initial lengths to the same length. This maneuver was performed in 8 strips from 6 dogs, after 30 min of carbachol stimulation and at 2 min intervals. At the end of each experiment, isoproterenol and atropine were added to the bath for concentrations of 1 mg/ml and 5 mg/ml. With the muscle thus relaxed completely, force-length curves were recorded for the full experimental range of muscle lengths without carbachol or AC stimulation.

**RESULTS**

When the isolated trachealis muscle was stimulated with AC at a constant length, force increased to a peak in about 8 sec and thereafter decreased, falling to between 30 and 80% of the peak force by 30 to 60 sec. Thirty to 60 sec stimulations, repeated at 10 min intervals, showed some diminution in the peak force developed, but the biphasic pattern during each stimulation was maintained. With the addition of carbachol to the isolated muscle, force increased over about 5 min and thereafter remained nearly constant. Within the range of $L_{sm}$ in this study, the parallel elastic component, (the $F_{sm}$ developed by the relaxed muscles) was less than 5% of the initial force developed with stimulation at 85% of $L_{sm}$.

![Graph 1](image1)

![Graph 2](image2)

**Fig. 5.** Isometric stress recovery after quick release (upper panel) and force immediately preceding release (lower panel) both plotted against duration of stimulation. Symbols as before. Those not connected were AC on the left and carbachol on the right. Rates were calculated as the isometric recovery in 2 sec divided by the initial change. The rate of recovery diminishes markedly with duration, although the initial force is not systematically changed.
Therefore, the forces developed by the muscle in this study can be considered as representing the active component.

**Series elastic component**

At various intervals after stimulation by AC or carbachol, quick stretch or quick release experiments were performed. Typical results are shown in Fig. 2.

We looked at the length tension behavior during the quick release or stretch to characterize the series elastic component (SEC). Striated (Jewell and Wilkie 1958) and smooth (Stephens and Kromer 1971) muscles behave as if they comprise an elastic component (SEC) in series with contractile elements (CE). Force-length tracings during the initial rapid length change, then, characterize the properties of the SEC. Typical oscillographic tracings of force change during (shown by dots) quick release are given in Fig. 3. These tracings cluster and the endpoints appear to describe a single curve. The quick stretches (not shown) describe a curve which appears continuous (though with increasing rather than decreasing force and length) with the quick-release curve shown. There appear to

![Graph showing rate of isotonic shortening and force immediately preceding release](image-url)

**Fig. 6.** Rate of isotonic shortening after quick release (upper panel) and force immediately preceding release (lower panel) both plotted against duration of stimulation. Symbols as before. The dotted lines connect data points for high dose carbachol. The velocity diminishes with duration of the stimulation despite constant or increasing initial force.
be no differences in the tracings obtained after 10 sec of AC stimulation and after 30 min of carbachol stimulation. The properties of the SEC, then, are not changed with increasing duration of stimulation.

**Quick stretch to isometric conditions**

When length was held constant after completion of the quick stretch maneuver (Fig. 2 left), force fell off gradually for more than 10 sec. We measured change of force during the first 2 sec of this isometric stress relaxation from the oscillographic tracings, expressed it as a fraction of the initial increase of $F_{sm}$, and plotted the results against the duration of stimulation (Fig. 4 upper). Solid lines indicate the stress relaxation after quick stretches performed 10 to 60 sec after the onset of AC stimulation. Dashed lines, indicate stress relaxation after quick stretches performed 1 to 30 min after the addition of carbachol. There are no substantial or systematic changes in stress relaxation with either duration or means of stimulation. The variability of stress relaxation is small compared to the effects of duration of stimulation on the rates of stress recovery and shortening velocity to be described below. Prestretch forces (Fig. 4 lower) were also unaffected by duration or means of stimulation.

**Quick releases to isometric conditions**

In analogous fashion, when length was held constant after the quick release was completed, (Fig. 2 middle) force increased towards the pre-release level. The rate of this isometric stress recovery was calculated from the oscillographic tracings as the force recovered during the initial two sec divided by the change of force.

![Fig. 7. Force ($F_{sm}$) during two sequential quick releases to the same length ($L_{sm}$) from different initial $L_{sm}$. At a given horizontal level, the muscle is at the same $L_{sm}$ and $F_{sm}$ but has very different rates of stress recovery. If the properties of the SEC are the same, then the velocity of shortening of the CE is reduced either by a greater duration of shortening or a shorter initial $L_{sm}$.](image)
which occurred with the quick release itself. Fig. 5 shows stress recovery in the upper panel and the pre-release force in the lower panel both plotted against the duration of stimulation. Again, the pre-release force does not vary systematically with duration. However, the rate of stress recovery diminished markedly with duration of AC or carbachol contraction.

Quick releases to isotonic conditions

If, after the quick release, the muscle was allowed to shorten at nearly constant force (Fig. 2 right), the length changed at a rate which was orders of magnitude slower than the initial shortening and was only slightly curvilinear over the first several sec. This isotonic velocity of shortening following quick release was plotted as the slope between 0.4 and 1.4 sec after the quick release divided by the length prior to release. Fig. 6 shows velocity in the upper panel and the pre-release force in the lower panel both plotted against duration of stimulation. The points for AC stimulation are again connected by solid lines and those for low-dose carbachol, $5 \times 10^{-8}$ g/ml are connected by dashed lines. The points for high-dose carbachol, $10^{-5}$ g/ml, are connected by light dotted lines. There would appear to be no systematic trend in the pre-release forces developed during AC stimulation or low-dose carbachol. Nonetheless, velocity decreases with duration of AC stimulation or low dose carbachol and indeed appears to be continuous between the two methods of stimulation. The velocity for high-dose carbachol in two animals also appears to be falling despite the fact that, if anything, the pre-release forces are increasing with duration of stimulation.

In order to look at the effect of length history, we performed quick releases to the same length from differing initial lengths. In Fig. 7, superimposed on each other are shown typical time courses during and after two quick releases. Both stress recoveries were made at the same length. There are grossly different rates of isometric stress recovery at the same length and force (compare the slopes of the force-time plots where the tracings are intersected by the horizontal dashed line). That is, the stress recovery at a given length and force is time-dependent, path-dependent, or both.

Discussion

Our major finding was that, after quick release, the rates of isometric stress recovery or isotonic shortening were markedly diminished as the duration of stimulation increased. The means of stimulation (electrical or cholinergic) did not seem to be involved. In contrast, stress relaxation after quick stretch did not change with duration.

Our analysis of these findings is based on the concept of A.V. Hill (1938) that muscle behaves as if it has elastic components in series with a contractile element. The existence of the series elastic component, SEC, in striated muscle is well accepted. The SEC has been thought to apply also in smooth muscle (Stephens
and Kromer 1971) on the basis of observations similar to ours and is characterized by the length-force behavior during quick releases and quick stretches. With quick release to a constant force (Fig. 2 right), the initial shortening, when plotted against developed force, follows a nearly consistent path regardless of the amplitude of shortening (Fig. 3). If the length is quickly returned to the original, a loop is described with little hysteresis (Sasaki and Hoppin 1979). Quick stretches from the initial length describe a path with similar initial slope as the quick releases from different initial lengths but with the same force (experimentally obtained by varying the stimulus to contraction). These properties seem consistent with a passive SEC. Our results suggest that the SEC was not affected by the duration of stimulation or the means of stimulation; the force-length relationship for the initial phase of shortening or lengthening appeared similar regardless of duration or means of stimulation (Fig. 3).

After the initial shortening or lengthening of the SEC was over, the strips showed isometric stress relaxation, isometric stress recovery, or isotonic shortening. These phenomena are thought to reflect the interaction of the elastic properties of the SEC and the force-velocity properties of the contractile element, CE. During quick stretches the SEC is lengthened. Following completion of the quick stretch, if length is held constant, the CE lengthens as the SEC elastically shortens and, as a consequence, force falls. The rate of this stress relaxation depends on the velocity of the lengthening of the CE and the passive elastic properties of the SEC. As shown in Fig. 4, the time course of stress relaxation was not substantially altered by the duration of the electrical or the carbachol stimulation. We conclude that the active contractile element, when lengthened, did not show any changes related to the duration of stimulation.

On the other hand, there was a dramatic decrease in the ability of the contractile element to shorten with increasing duration of stimulation. After completion of quick release, the CE shortens actively. Under isotonic conditions, the shortening of CE is seen as shortening of the muscle strip and under isometric conditions the CE shortening at the expense of SEC lengthening appears as stress recovery. Fig. 5 shows that isometric stress recovery markedly diminished with increasing duration of stimulation and Fig. 6 shows that shortening velocity decreased whether the muscle was stimulated by AC or low-dose carbachol. Our two results for high-dose carbachol also suggest dependency on duration of stimulation. The initial force prior to quick release was generally the same for all the studies with AC and low-dose carbachol stimulation. The apparently continuous relationship of shortening velocity and duration of stimulation, then, suggests the generalization that the differences are simply due to the duration of stimulation.

One possible explanation for the findings would be a change in the underlying force-length relationship with duration such that the force at 85% $L_{sm}$max was maintained but forces at lower $L_{sm}$ were reduced. To rule out this possibility, we
studied 3 strips from two dogs at initial $L_{sm}$ of 60% $L_{sm\max}$. We again observed maintainance of initial $F_{sm}$ and decrease of velocity of shortening with the duration of active state and concluded that the change in the velocity of shortening was not simply due to a major change in the slope of the force-length characteristic of the CE. The mechanism of time dependent reduction in shortening velocity with increased duration of stimulation has been demonstrated by Dillon et al. (1981) and Gerthoffer and Murphy (1983). These investigators observed that the force-velocity curve of the muscle became progressively depressed during the course of a tonic contracture by K$^+$ solution or carbachol. The rate of myosin phosphorylation decreased concurrently, suggesting that the rate of cross bridge cycling was declining with extended periods of contracture. We confirmed that shortening velocity during either AC or carbachol stimulation was dependent on the duration of stimulation.

Parenthetically, note should be made of the complexity of the mechanical properties of this system. As noted above, the Hill model suggests an analysis of isometric stress recovery following quick release wherein the CE shortens at the expense of the SEC. We reasoned that if the SEC had constant elastic properties and the CE had simple length-force-velocity relationships, then one would anticipate the same rate of stress recovery at the same $L_{sm}$ and $F_{sm}$ regardless of the time-path history to that point. Accordingly we performed quick releases to the same $L_{sm}$, but from different initial $L_{sm}$ (Fig. 7). The initial $F_{sm}$ were nearly the same because the differences in $L_{sm}$ were small compared to the length of the muscle and because the general force-length characteristic is highly compliant in that region. The forces at the same $L_{sm}$ after quick release, however, differed because of the different magnitudes of change of length. With stress recovery, within a few seconds the force in the lower curve came within the range of the upper curve. The rates of stress recovery (slopes) at the same $L_{sm}$ and the same $F_{sm}$ (horizontal line in Fig. 7 for example) differ markedly; the curve with the smaller amplitude quick release and shorter interval after quick release has the greater rate of stress recovery and, by the Hill model, the greater velocity of shortening of the CE. The CE, then, shows prominent time or path-dependency after quick release. This observation does not challenge the validity of our previous conclusion that the duration of stimulation affected the ability of the CE to shorten, as the quick releases on which those conclusions were based were performed with similar length changes and timing.

When the trachealis muscle was stimulated with AC, we consistently found a response of the isometrically developed force in which a fall commenced about 8 sec after the onset of the field stimulation. We confirmed that the response to electrical stimulation was almost completely blocked by adding atropine (5 mg/ml, 2 strips) to the bath (Stephens and Kroeger 1980). Stephens and Kroeger (1980) examined this phenomenon and concluded that the supramaximal AC stimulation may be depleting cholinergic nerve terminals. By the contrast, when
stimulated by the cholinergic agent carbachol, the increase of isometrically measured force occurred over several min and then was maintained over a period of an hr or so. Siegman et al. (1976) showed that ATP consumption was almost nil during periods in which force was maintained for a considerably long time. These non-ATP-utilizing force-sustaining cross-bridges have been termed “latch bridges” by Dillon et al. (1981).

Our major finding, reduced contractile performance with increasing duration of stimulation, is not related to the differences in means of stimulation, which must be considered whenever studying the dynamic responses of smooth muscle. For example, in our previous study (Sasaki and Hoppin 1979), smooth muscle chronically constricted with carbachol showed time-dependent phenomena which were more dramatic than those observed with electrical stimulation at a shorter duration of the stimulation. If such differences hold up in situ, then there may be differences which have physiological relevance to the intact animal. For example, if the time-dependent behavior of contracted smooth muscle changes with duration of contraction, then the effects of that time-dependency on forced expirations (Green and Mead 1974) and on airway caliber (Sasaki and Hoppin 1979) may differ in acute challenges and prolonged bronchoconstriction or in bronchoconstriction due to vagal reflexes and mediators (Hida et al. 1984). For another example, with SO₂ inhalation (mediated by the vagus), airway resistance at functional residual capacity (FRC) is increased and is not decreased by a deep inflation to TLC whereas after histamine inhalation, airway resistance is increased but can be decreased by a deep inflation to total lung capacity (TLC) (Jere Mead, personal communication). The explanation could be that the vagally mediated response to SO₂ involves shorter stimuli than the response to histamine. Boushey et al. (1980) suggested that the force-velocity relationship would provide useful information concerning the dynamic properties of bronchial hypersensitivity. Antonissen et al. (1979) studied the force-velocity relationship of the trachealis muscle of the canine asthmatic model. They observed the increased shortening velocity of sensitized animals. We reported force-velocity relationship of the trachealis muscle of the dog during vagus nervous stimulation (Okayama et al. 1982). The present mechanical properties of trachealis muscle should be considered for these measurements of force-velocity relationship.

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References


