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EFFECT OF HISTAMINE ON THE LENGTH–TENSION PROPERTIES OF GUINEA PIG TRACHEALIS MUSCLE[1]

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Abstract. The isotonic length–tension properties of excised live guinea pig trachealis muscle have been determined as a function of histamine concentration. Over the range of histamine concentrations studied, a family of curves corresponding to the total and active tensions were obtained. Reciprocal plots of the tension–concentration data revealed a length dependence of the affinity constant of histamine for its receptor The tensions and lengths below resting were considered to be of special importance because of the role of muscle contraction in airway closure. A mechanochemical model for shortening and re-elongation of the trachealis muscle during anaphylaxis has been proposed on the basis of this data.

By comparing the force–length properties of the muscle at the lowest histamine concentration, it was suggested that the formation of a histamine-Ca^{++} receptor complex causes an increase in the rigidity of the muscle membrane. The complete range of lengths, tensions, and concentrations must be employed in order to propose a mechanism of action from mechanical data alone.

Airways Length–tension properties
Histamine Trachealis muscle

Considerable data have been compiled concerning the effects of histamine on smooth muscle. Besides its frequent use in isometric experiments as a standard agonist, histamine is considered to be one of the major mediators released during allergic and anaphylactic reactions (Austen, 1974) in guinea pig (Bartosch *et al.*, 1932) and possibly human lung (Brocklehurst, 1960; Kaliner *et al.*, 1972; Schild *et al.*, 1951). In guinea pig lung, this contributes to the resulting bronchospasm (Brocklehurst and Lahiri, 1962; Piper and Vane, 1967), the increase in airway resistance, and the reduction in lung compliance (Nadel, 1973). These effects are a result of shortening

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of the respective smooth muscles (Dale, 1913; Shultz, 1910). Consequently, it would be of primary importance to understand the mechanical properties of the airway smooth muscle as a function of length and concentration of histamine (Joiner *et al.*, 1974). Isometric studies on smooth muscle have provided histamine dose–response data at fixed lengths (Nickerson, 1956; Rocha e Silva, 1959; Weiss *et al.*, 1975), but the full range of length–tension as a function of histamine concentration has not been evaluated. In order to elucidate the mechanism of action of histamine in contracting airway muscles, the model must include the complete mechanochemical properties of the muscle, especially at the shortened lengths because it is this state which simulates the progressive degrees of bronchial muscle contraction. This report, therefore, will concentrate on the effect of histamine on the tension developed by guinea pig trachealis muscle at lengths below resting.

Method

The trachea was excised from adult albino guinea pigs as previously described (Weiss *et al.*, 1975) and cut into cylinders of about two cartilage rings per segment. Each segment was cut opposite the trachealis muscle and clamped on the cartilage at both ends with alligator clamps as shown in fig. 1. The muscle was oriented so that the fiber bundle axis was parallel to the direction of length displacement. The isotonic apparatus used in these experiments was similar to that used for glycerinated psoas (Hargraves and Mandelkern, 1977) with some modification because of the extremely short lengths of the guinea pig trachealis muscle. The Harvard Model 382 Rotary Motion Transducer, used for the measurements of length, was fitted with a lightweight rod which served as a lever to provide the mechanical advantage necessary for the isotonic experiments. One end of the muscle was attached to the rod by a hook and alligator clamp which was free to move as weights were added to the opposite end of the rod. The other end of the muscle was clamped so as to be immobilized in a Harvard Muscle Warmer # 317 into which 30 ml of Krebs–Henseleit solution could be added. The solution was buffered at pH 7.4 by continuously bubbling 95% O_2–5% CO_2 mixture throughout the experiment. The temperature was maintained at 37.0 ± 0.1 °C by immersing the muscle warmer in a thermostatted water bath. Care was taken at the beginning of each experiment to exactly balance the clamp and rod by adding weights on the opposite end such that there would be zero force on the muscle when attached. On shortening, the signal from the transducer was amplified by the Harvard Model 362 Amplifier and recorded with the Hewlett-Packard Model 680 Strip Chart Recorder set to 100 mV range. The chart was calibrated from 0.0 to 0.5 mm full scale on this range. The electronic noise level was about 0.01–0.02 mm which causes an uncertainty in the measurement of length of about 2%.

The initial length of the muscle (L_0) is defined as the length of the muscle under zero force when the cartilage rings are cut opposite the muscle (fig. 1). After equilibration at room temperature for 10–30 minutes in Krebs–Henseleit, L_0 was measured

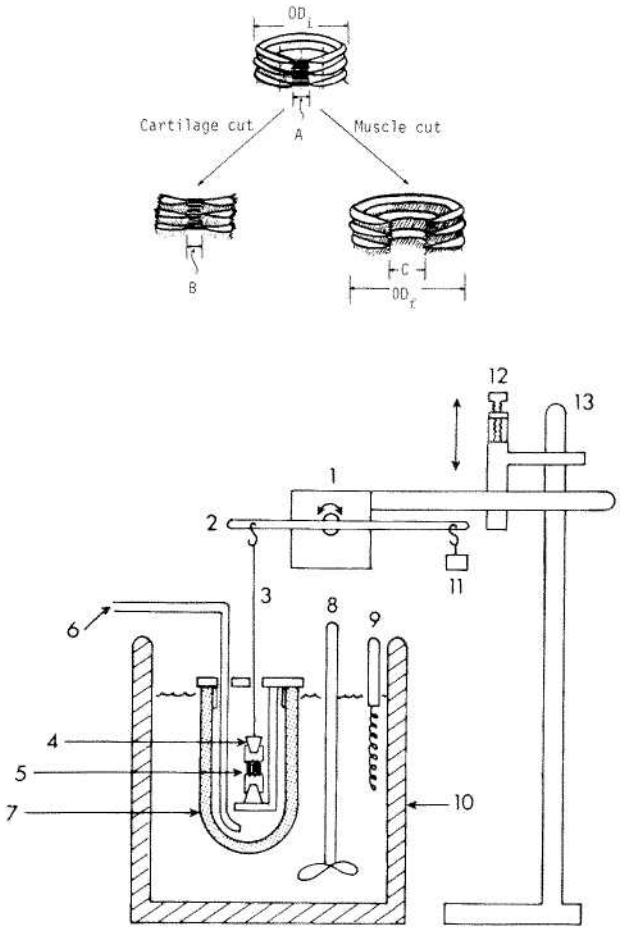

Fig. 1. Schematic of the isotonic apparatus and the manner in which trachealis muscle was cut in order to make measurements recorded in table 1. OD_i = initial outer diameter before cutting muscle; OD_f = final outer diameter after cutting muscle; A = length of intact muscle; B = length of muscle after cartilage was cut; C = length between cartilage after muscle was cut; 1 = rotary motion transducer (Harvard # 382); 2 = aluminum rod; 3 = stainless steel wire; 4 = alligator clamp; 5 = trachealis muscle; 6 = CO_2 : O_2 inlet; 7 = sample warmer (Harvard # 317); 8 = stirrer; 9 = thermostat (immersion heater); 10 = water bath; 11 = weight; 12 = adjustable clamp (Harvard # 214); 13 = ring stand.

with a Vernier caliper which is capable of measuring length to within 0.1 mm. The values for L_0 determined by this method ranged from 0.5 to 1.0 ± 0.1 mm.

After the initial length of the muscle segment was measured, mounted in the warmer, and attached to the transducer, it was allowed to equilibrate at 37 °C for 10–15 minutes in the Krebs–Henseleit until a stable base line of 0.0 to ± 0.02 mm/min was obtained. At this point, the appropriate concentration of histamine was injected into the Krebs–Henseleit solution via a 1.0 ml syringe. After injection, the muscle started to shorten within 5 seconds and maximal shortening at zero load was observed

within 3 minutes. The muscle was then elongated by the addition of increasing milligram weights to the opposite end of the lever arm. After the addition of each weight, the corresponding change in length was stable after about 1–2 minutes. The experiment was terminated either when the muscle had been stretched to the region of high modulus (about twice its initial length) or if the response to stretch caused myofilament shortening. The myogenic response when it occurred was minimal compared to that for taenia coli (Stephens *et al.*, 1975) and was not observed until the muscle had been stretched to a relative length greater than 1.5. On completion of the experiment, the muscle was dissected from the cartilage, dried, weighed, and the cross-sectional area determined (Gordon and Siegman, 1971). The tension was then expressed as kg/cm^2 which included the necessary corrections for the decreasing cross-sectional area during muscle elongation. Active tension was calculated as the difference between the total tension obtained with a maximal histamine concentration and that obtained from a histamine concentration below the threshold for muscle shortening.

Approximation of *in vivo* resting length of the trachealis muscles was made in order to compare it with L_0 and to assess the significance of L_0. Various measurements were made and are summarized in table 1 and fig. 1. Column A refers to the measured length of the trachealis muscle before the cartilage is cut; B, after the cartilage is cut ($B = L_0$); C, the gap between the ends of the cartilage when the muscle is cut (should equal $A - D$; however, this did not occur experimentally due to the difficulty in determining the muscle boundary); D, the difference in outer diameter of the trachea before and after the muscle is cut ($A + D = L$). The numbers in the column labeled 'L/L_0' should represent the upper limit of the *in vivo* relative resting length of the muscle since the maximum length, L, is determined by the recoil of the cartilage. That is, the muscle can be no longer than the trachealis cartilage can stretch it when not exposed to the negative intrathoracic pleural pressure.

An approximation of the normal operating range of lengths can also be obtained by determining the limits of reversibility (Peterson and Paul, 1974). The limit of reversibility for the various concentrations of histamine was determined by measuring length at zero force, loading the muscle, allowing it to elongate and measuring the length at zero force again after removing the load. This was done by removing all weights simultaneously and by removing them sequentially. If the muscle returned to its original length ($\pm 2\%$) at zero load, the deformation was considered to be reversible. The data was not analyzed for hysteresis since elongation followed the same path on the second and subsequent trials.

Results

At 37 °C and zero force, the trachealis muscle exhibits a concentration dependent shortening to about 50% of its initial length (fig. 2). Similar amounts of shortening have been observed by Stephens (Stephens *et al.*, 1969) for dog trachealis muscle.

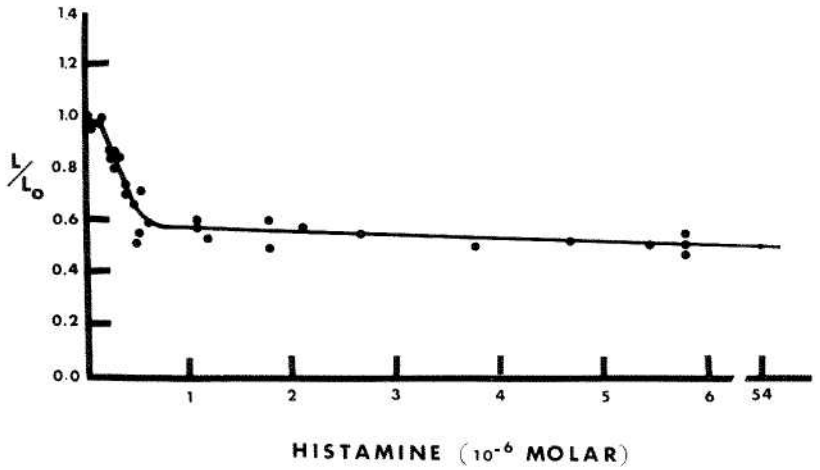

Fig. 2. Relative length of trachealis muscle as a function of added histamine at zero force and 37 °C (solvent was Krebs–Henseleit, pH = 7.4).

At concentrations below 0.25×10^{-6} molar no observable changes in length occur. However as the concentration of histamine is increased to 0.5×10^{-6} molar a near maximal change in length is observed. The shortening is cooperative, that is it occurs over a small range of histamine concentration ($0.25–0.5 \times 10^{-6}$ M). As the concentration is increased above 0.5×10^{-6} M only a small amount more shortening occurs. The transition from resting length to a highly shortened state will be used as reference curve from which the deformation is initiated.

If the trachealis muscle is stretched from a point at which it has been maximally shortened (*e.g.* 5.4×10^{-5} M histamine), it will follow a curve (fig. 3) qualitatively similar to that observed for glycerinated psoas muscle (Hargraves and Mandelkern, in press). However, on a quantitative basis, the modulus or slope of the curve at all lengths is much less and the inflection point occurs at a lower force. As the concentration of histamine is lowered from 5.0×10^{-5} to 0.5×10^{-6} and stretch initiated at this point, the shape of the length-tension curve is altered and the inflection point occurs at a lower force (fig. 3). It is these length changes at the lower forces which have not been observed in the past and are crucial to the understanding of muscle contraction (Mandelkern, 1971) (Rubin *et al.*, 1969). If the concentration of histamine is lowered further to less than 0.5×10^{-6} M (above the mid-point of the curve in fig. 2), the length-tension properties are more characteristic of passive tension. It is interesting to note that the muscle in Krebs–Henseleit solution is more easily deformed than it is in 0.25×10^{-6} M histamine. As can be seen in fig. 2, this concentration of histamine is below threshold for initiation of shortening and no differences were observed at zero force. Upon initiation of a force, however, the differences have been uncovered. This implies that histamine may have some effect on the elastic properties of the membrane and/or interstitial components along with its

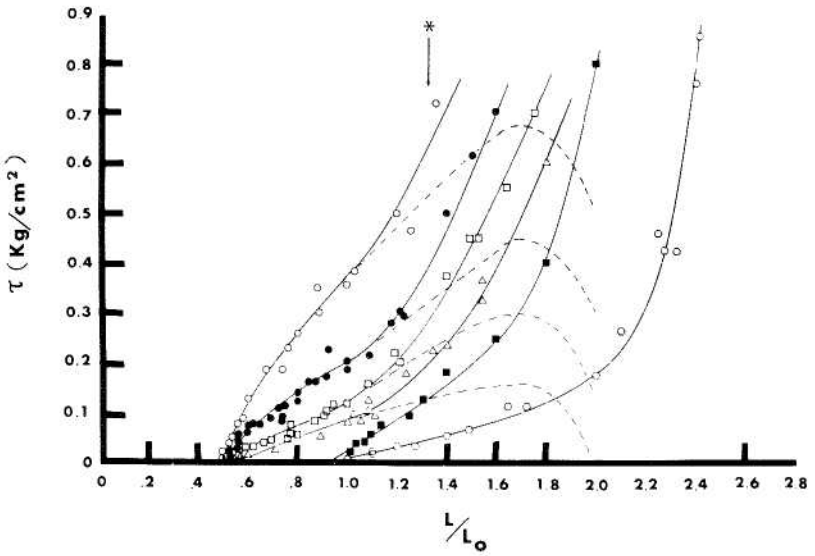

Fig. 3. Total tension in Krebs–Henseleit as a function of relative length at various histamine concentrations. ○ = 5.4×10^{-5} M; ● = 4.6×10^{-6} M; □ = 1.7×10^{-6} M; △ = 0.54×10^{-6} M; ■ = 0.25×10^{-6} M histamine (passive); ▯ = 0.0 histamine. (- - -) = corresponding active tension with 0.25×10^{-6} M histamine concentration as reference (data obtained by extrapolation of slope at highest tensions). * = maximum *in vivo* length.

TABLE I

Length measurements on trachealis muscle [a]

Trial	A	B (L_0)	C	OD_f	OD_i	$\frac{D}{OD_f - OD_i}$	L/L_0
1	0.9	0.9	0.9	3.9	3.5	0.4	1.44
				4.1	3.5	0.6	1.66
2	1.0	0.9	1.1	4.2	3.8	0.4	1.55
3	0.7	0.8	1.2	4.1	4.0	0.1	1.00
4	0.6	0.7	1.6	4.0	3.6	0.4	1.42
5	0.8	0.6	1.6	4.3	4.1	0.2	1.66
6 [b]	0.9	1.0	1.5	4.5	4.4	0.1	1.00
7 [b]	1.1	0.9	1.6	4.0	3.8	0.2	1.44
	1.1	0.9	1.6	4.4	4.1	0.3	1.55
8 [c]	0.5	0.5	1.4	4.7	3.9	0.6	2.2
9 [c]	0.5	0.5	1.3	4.4	3.5	0.9	2.8
							Average = 1.41 [d]

[a] Length in mm ±0.1 mm, columns defined in text and fig. 1.

[b] Measurements made immediately after excision.

[c] Partially contracted muscle; measurement made approximately 6 hr after excision.

[d] Trials 8 and 9 not included.

known effects on the contractile elements. Because of this possibility, it seemed that the low concentration curve should be used as the passive reference in the calculation of the active tension (interrupted lines in fig. 3). The maximum in the active tension obtained at the various histamine concentrations ranged from 0.67 to 0.15 kg/cm^2. These concentrations of histamine provide active tensions which fall within the range of that determined for canine trachealis (1.1 kg/cm^2) (Stephens *et al.*, 1969) and canine bronchus (0.20 kg/cm^2) (Stephens *et al.*, 1968). Most other smooth muscles also fall within this range (Gordon and Siegman, 1971) with glycerinated uterus providing the lowest value of 0.06 kg/cm^2 (Hasselbach and Ledermair, 1958).

Conventional active tensions (difference between histamine total and K–H passive curves) can be taken from fig. 3 at various constant lengths and plotted as tension versus concentration to provide the usual isometric dose–response curves as shown in fig. 4. It can be seen that each length has a characteristic dose–response curve with

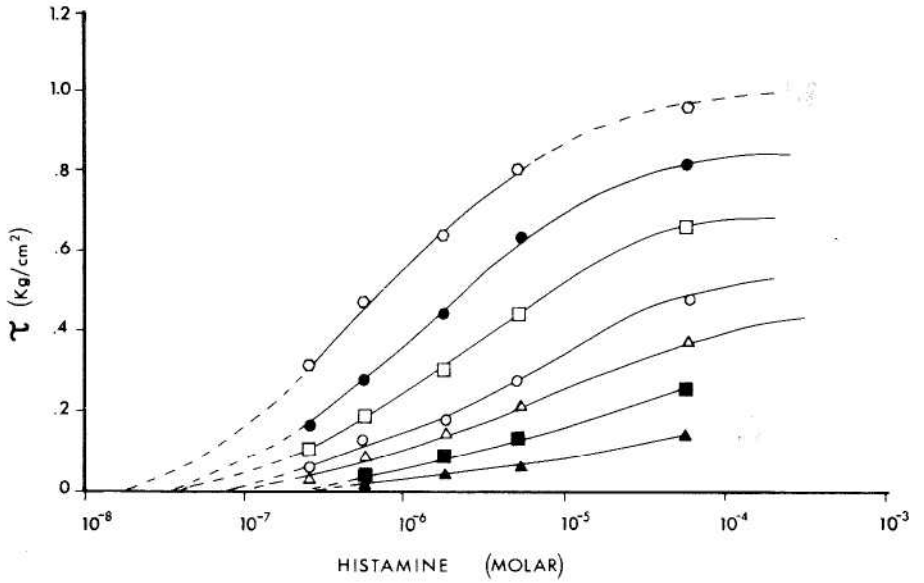

Fig. 4. Data of fig. 3 replotted as dose-response at various relative lengths. ◌ = 1.8 L/L_0; ● = 1.6 L/L_0; □ = 1.4 L/L_0; ○ = 1.2 L/L_0; △ = 1.0 L/L_0; ■ = 0.8 L/L_0; ▲ = 0.6 L/L_0.

the maximum depressed as the length decreases from 1.8 to 0.6 L/L_0. It should also be pointed out that the ED_{50} (concentration at half maximal tension) is shifted towards higher histamine concentration as the length decreases to L/L_0 of 1.0. The concentrations below the ED_{50} at the longer lengths are primarily composed of tension which arise from changes in passive properties of the muscle. The dose–response data at longer lengths is, therefore a mixture of passive and active tensions as noted in fig. 3.

The data of fig. 4 can be analyzed in the usual manner by plotting the reciprocals

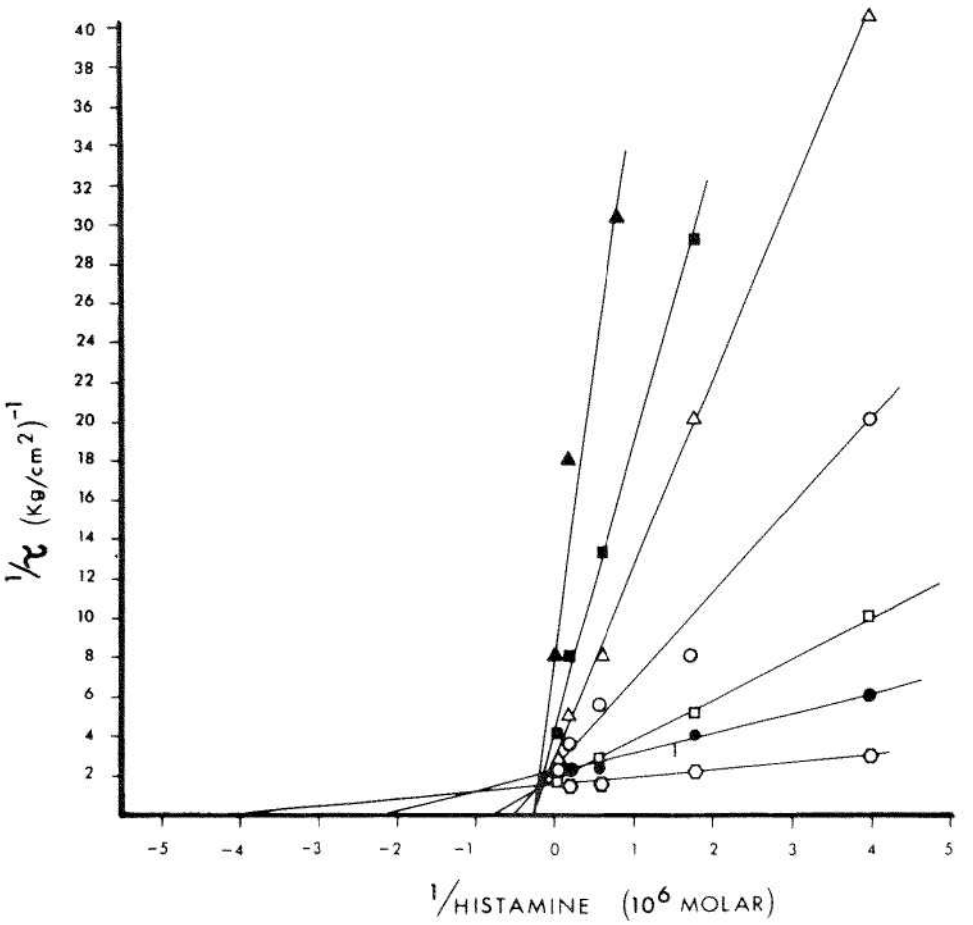

Fig. 5. Reciprocal analysis of data in fig. 4 symbols refer to lengths described by fig. 4.

of the tension and concentration. If this is done it can be seen that according to fig. 5 a linear relationship is obtained. From the intercept of the abscissa, the affinity of histamine for its receptor can be estimated. The binding constant at a length of 1.8 L/L_0 was found to be 4.0×10^6 L/mole which is in agreement with literature values of $4.2–5.0 \times 10^6$ L/mole (Rocha e Silva, 1959; Nickerson, 1956). However, it can be seen that this value decreases as the length decreases to 1.0 L/L_0 where it remains at a constant value of 3.0×10^5 L/mole. Apparently the affinity can vary over 10-fold depending upon the length chosen for the isometric experiments.

Since the changes in the passive elastic properties of the trachealis muscle, at low histamine concentrations might be considered an unusual observation, further data were obtained to substantiate this phenomenon. Separate experiments were conducted in which a muscle was elongated in Krebs–Henseleit and subsequently allowed to return to its initial length by removing all the weights. At this point histamine was

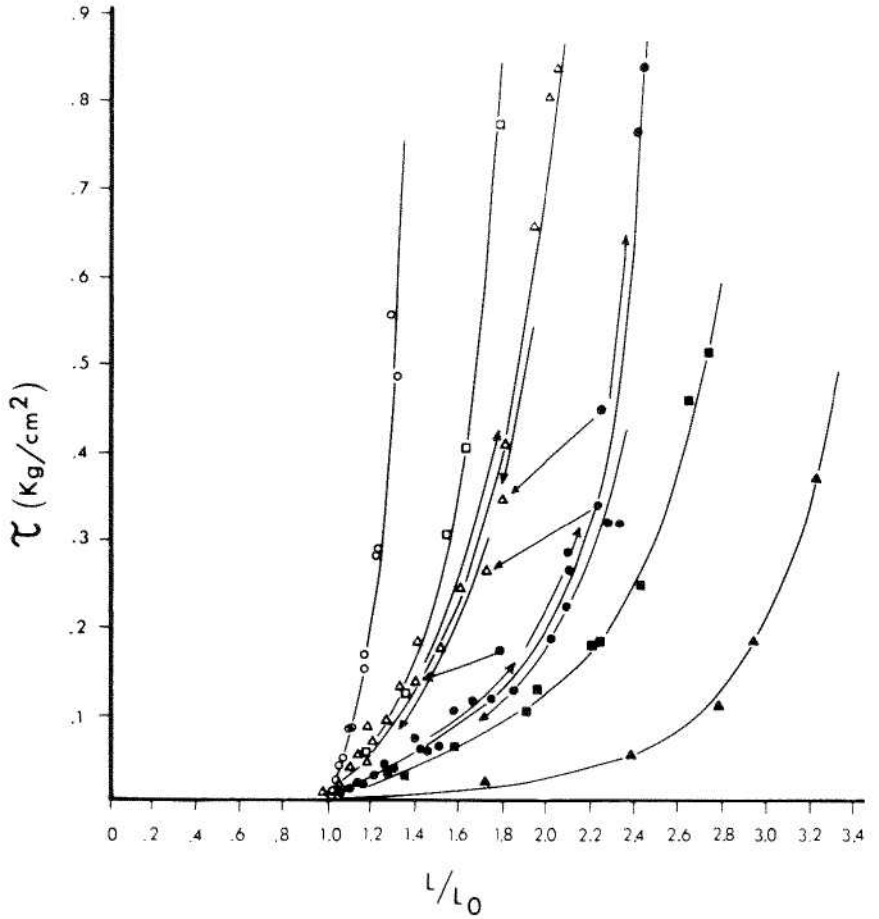

Fig. 6. Passive tension as a function of length. ○ = glycerinated muscle (as described in ref. 17); □ = Brij-treated muscle (according to ref. 43); △ = K–H + histamine (0.25×10^{-6} M); ● = K–H (2.52 mM $CaCl_2$) alone, ■ = K–H no Ca^{++} added, ▲ = epinephrine (2.7 μM).

added to the bath to a concentration of 0.25×10^{-6} mole and the muscle reelongated. This experiment was repeated several times on various fibers and the results are described by the path of the arrows in fig. 6. Other experiments described by arrows connecting separate curves were performed in which histamine (0.25×10^{-6} molar) was added following stretch in Krebs–Henseleit to 2.2 and 1.8 L/L_0. The muscle stimulated with histamine at this point shortened very slowly (5–10 minutes to reach maximum) to a point which falls on the curve described by other muscles treated with 0.25×10^{-6} M histamine initially. These data clearly establish that there are differences in the passive elastic properties of the trachealis muscle even at very low histamine concentrations. These changes were not observed, however, when the Ca^{++} in the Krebs–Henseleit was removed before the muscle was treated with 0.25×10^{-6} M histamine. Stretching of muscles in epinephrine – KH, 0.0 Ca^{++},

Brij-treated and glycerol-extracted muscles in K–H also perturbed the elastic properties in the various ways shown in fig. 6. It appears that removing the membrane with glycerol extraction and Brij detergent results in increased rigidity of the muscle, whereas removing Ca^{++} or addition of epinephrine causes the muscle to become less rigid.

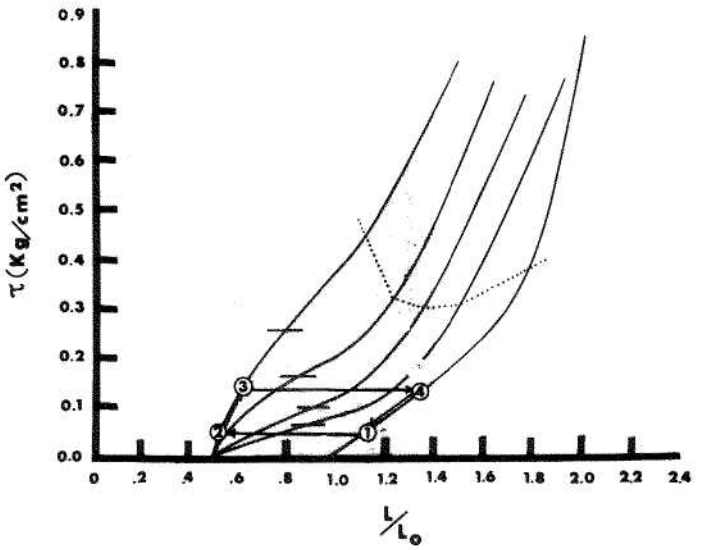

Fig. 7. Data of fig. 3 replotted to demonstrate proposed cycle of shortening and re-elongation (1 → 2 → 3 → 4). Dotted line represents limit of reversible length changes. Shaded area represents proposed normal operating limits of trachealis muscle.

The dotted line in fig. 7 represents the average upper limit of reversibility ($0.5 < L/L_0 < 1.8$) for the trachealis muscles used in these experiments. This limit is similar to that determined previously (Peterson and Paul, 1974) and occurs near the resting length of the muscle. The data in table 1 indicate that the *in vivo* resting length of the muscle probably does not exceed 1.4 times the reference length defined here as $L/L_0 = 1.0$. The *in vivo* resting length for the guinea pig trachealis is approximately 76% of the length at maximal active tension, a result similar to that found for guinea pig taenia coli (Mashima and Yoshida, 1965) and implies that these muscles probably do not normally function at the maximum in the curve.

Discussion

The mechanical properties of muscle are usually characterized by measuring the length changes which accompany deformations from previously specified forces. The measurements can be made with and without stimulation at many forces in order to define total and passive tensions, respectively. The passive tension which

results primarily from the membranes and interstitial components can be subtracted from the total tension and a resulting active tension corresponding to the activated myofilaments is obtained. The active tension has been usually considered to be the important state of the muscle upon which most interpretations are based. Recently, however, A. V. Hill's concept of an 'active state' was questioned because of changes in the intensity and time course of the active tension (Julian and Moss, 1976). Since the initial length as well as changes in length can also affect the active state, the following discussion will focus primarily on the total and passive tension properties.

The length–tension relationships for smooth muscle following agonist challenge have been generally obtained at muscle lengths *greater* than resting (Aberg and Axelsson, 1965; Brady, 1967; Gordon and Siegman, 1971), and then only the active tension considered. This is not entirely physiological, since for airway smooth muscle under conditions of bronchospasm, the significant lengths are those in which the mucle was contracted or shortened. It is therefore important to approximate the *in situ* or normally operating length range of such muscles. From the measurements summarized in table 1 and the measured limit of reversibility in length, it appears that the relative resting *in vivo* length is something less than 1.4 L/L_0. Therefore, the functional range of the guinea pig trachealis muscle normally, and during induced bronchoconstriction, is likely to be contained within the shaded area of fig. 7.

The combined data of figs. 2, 3 and 6 demonstrate that force–length measurements can be used in contractile systems to distinguish contractile events occurring at the membrane level from contractile events occurring within the myofilaments. This observation would not be possible if the data were obtained isometrically, *i.e.* at only one length. Low concentrations of histamine cause changes in the compliance of the system without causing any shortening of the myofilaments at zero force (as can glycerination, Brij, 0.0 Ca^{++}, and epinephrine). Regardless of the initial tension the muscle will not shorten below L/L_0 of 1.0 unless the myofilaments have been activated. Therefore all data in fig. 6 can be interpreted in terms of changes in the membrane or interstitial components which result in a change of the overall elastic properties without initiating active shortening of the myofilaments. In the case of histamine the increase in rigidity of the system (fig. 6) most likely results from either (1) opening of Ca^{++} channels in membrane and thereby exposing myofilaments to the supernatant concentrations of Ca^{++}, (2) increasing the amount of Ca^{++} bound to membrane as in a histamine–Ca^{++}–receptor complex, or (3) initiating a conformational change in membrane components. The experiments with Brij and glycerol treated fibers tend to favor hypothesis number (1), since both agents are known to remove membrane components (Hargraves and Mandelkern, in press; Orenthecker *et al.*, 1974). This treatment exposes the myofilaments to the high Ca^{++} (2.5 mM) in the Krebs–Henseleit medium which can increase the crystallinity of the myofibrils (Hargraves and Mandelkern, in press).

It is interesting, however, that Ca^{++} binding alone can increase the rigidity of membrane systems (Sauerheber and Gordon, 1975) and perhaps a histamine–Ca^{++}–receptor complex may do the same. It has been demonstrated that histamine binding

to its receptor requires Ca^{++} (Takayanagi *et al.*, 1974) and the data here also suggests that Ca^{++} is necessary for this change to occur since no changes in the fluidity of the system were observed after adding histamine to 0.0 Ca^{++} K–H. Furthermore, it has been reported that norepinephrine causes an increase in extracellular binding of Ca^{++} but does not cause any increase in Ca^{++} influx (Van Breemen and Lesser, 1971). It is possible that histamine follows a similar mechanism. The third alternative may result from or be concurrent with binding but no evidence can be presented which would favor this mechanism alone.

The observation of decreasing binding affinity (or increased dissociation) with decreasing length as seen in fig. 5 does not seem to be consistent with the opening of Ca^{++} channels but might be rationalized on the basis of the membrane bound histamine–Ca^{++}–receptor complex. At the shortest lengths, localized concentrations of the charged complex resulting from the highly evaginated membrane (Fay *et al.*, 1976) can cause electrostatic repulsion which will decrease the affinity of the receptor for histamine. On the other hand when the muscle is stretched the receptors may be separated by larger distances and the affinity of the receptor for the histamine–Ca^{++} will be optimized. Therefore the data appear to favor the idea that a histamine–Ca^{++}–receptor complex is formed which causes an initial increase in rigidity of the membrane. This event is probably followed by release of the intracellular transmitter on the inner side of the membrane (Rocha e Silva, 1966).

It has been suggested by Stephens *et al.* (1968, 1969) that trachealis muscle provides a good model for studying the properties of airway smooth muscle. Active length–tension data was obtained by these workers using electrical field stimulation and it was used to explain various aspects of airway closure. Mashima and Yoshida (1965), however, have shown that the length–tension properties of spontaneously contracted guinea pig taenia coli are very much different from that obtained by field stimulation. Field stimulation may then be limited in the analysis of normally contracting and anaphylactic smooth muscle because the latter events are chemically mediated phenomena.

A cycle of shortening and re-elongation of a trachealis muscle may be envisioned using the data of fig. 7. Assuming the resting muscle at a length and tension depicted by state 1, an increase in agonist concentration such as histamine would cause the muscle to shorten at constant tension to length 2. The cartilage, however, exerts a force on the fiber and will continuously elongate it to length and tension state 3. The exact position of state 3 on the curve however, will be determined by the compliance of the cartilage. If the cartilage is very stiff, state 3 will be displaced towards higher tension and the magnitude of the length change will be decreased. In actuality the muscle will go directly from 1 to 3. At the lower histamine concentrations shown in fig. 7 it can be seen that only a small variation in the compliance of the cartilage can result in a large change in length. Furthermore, depending upon the ratio of cartilage to muscle, it is also possible for the muscle to shorten at constant tension. This may be important in the lower airways where cartilage is so sparse that on shortening from agonist stimulation the tone of the muscle must remain unchanged,

thereby preventing collapse of the airway. Based on the cycle presented in fig. 7, this would correspond to a reversible isotonic transformation of myofibrils in the muscle from state 1 to state 2 in contrast to the transformation from state 1 to 3 for the muscles of the upper airways.

These data suggest a possible role for the seemingly inert cartilage. If the compliance of the cartilage is abnormal, it can modify the tension on the muscle and prevent its return to the resting state; this may predipose to airway closure (Croteau and Cook, 1961).

Enzymatic conversion and removal of histamine can effectively reduce its concentration and the muscle will spontaneously re-elongate to length and tension state 4. When the recoil of the cartilage is no longer exerted on the muscle, the muscle can return to its resting state. This cycle demonstrates that it is the *total* tension and agonist concentration at the shortened lengths, rather than the active tension at lengths longer than resting which must be considered when interpreting length changes in tracheal muscle. Interpretations based upon active tension where only one length–tension curve is being considered depend upon muscle shortening by a decrease in the external force on the muscle (Stephens *et al.*, 1968). That is, in order for the muscle to shorten, the force must decrease. It is completely unclear as to how this could happen *in vivo*. It is, furthermore, dependent upon the passive tension which is not a factor in working muscle at lengths less than 1.0 L/L_0.

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