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Dependence of the Length–Tension Relationship on Agonist Concentration in Vascular Smooth Muscle (42371)

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Abstract. This study examines the dependence of the length–tension (L–T) relationship in vascular smooth muscle on its level of activation. A horizontal shift of the L–T relationship with a change in activation level has been shown in striated muscle when L–T curves could not be superimposed. Active force at each length was normalized to the maximum active force in each curve. Indices of a horizontal shift of a L–T curve include the initial length for an active response (L_i) and the length for maximum active force (L_{max}). In this study normalized L–T curves were obtained from rings of the dog anterior tibial artery at low (approximately ED_{50}) and high (maximal activation) concentrations of potassium (K^+), norepinephrine (NE), and calcium (Ca^{2+}). The normalized curve with a low concentration of K^+ or NE was shifted to the right of the curve obtained with a high concentration. L_i and L_{max} were significantly longer for a low concentration of K^+ or NE than a high concentration. With the same concentration of NE (10^{-5} *M*) no difference in the normalized L–T curves, in L_i, or in L_{max} were found when low (0.085 m*M*) Ca^{2+} experiments were compared to normal (1.7 m*M*) Ca^{2+} experiments. It may be concluded that the length–tension relationship in vascular smooth muscle is shifted to longer lengths with a decrease in the concentration of a chemical agonist but not by a decrease in external calcium. We suggest that a concentration dependent shift in the length–tension relationship may have a role in the regulation of blood flow.

The length–tension relationship has been considered to be mechanical evidence for the sliding filament model in muscle. However, recent studies have shown that this relationship is dependent on additonal factors (1). Studies on striated muscle show that intact fibers produce proportionally less force than chemically skinned fibers when length is decreased from its maximum response length (L_{max}) (2, 3). In these studies the force at each length is normalized to the maximum force in the curve. As a result the length–tension curve from intact fibers is shifted to the right (longer lengths) of the curve from skinned fibers. This indicates that intact fibers produce less force than the contractile mechanism is capable of generating at lengths less than L_{max}. Other investigators have shown a horizontal shift of the length–tension curve from intact heart muscle (4, 5) and from intact skeletal muscle (6) when the degree of activation is changed (i.e., changes in external calcium and other inotropic agents and in the pattern of electrical stimulation). It is generally agreed that length affects the activation process of striated muscle as well as the overlap of filaments.

For smooth muscle Arner and Hellstrand (7) have shown that intact specimens produce proportionally less force than chemically skinned specimens at lengths less than L_{max}. The effect of changes in activation level on intact vascular smooth muscle and its length–tension relationship has not been investigated. However, from norepinephrine dose–response experiments at various lengths we proposed that certain characteristics of the length–tension relationship may depend on the concentration of agonist (8). When the responses to a high agonist concentration from the various lengths were compared a length for maximum response (L_{max}) could be obtained from the dose–response data. At a low concentration the response appeared to continually increase with length. This suggests that L_{max} is shifted to longer lengths as agonist concentration is decreased. Other reference lengths such as the initial length for an active response (L_i) could be similarly affected. Changes in these characteristic lengths would occur if the length–

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tension relationship were shifted to a different range of absolute lengths. This raises the possibility that a change in agonist concentration may modify the role of the length–tension relationship in blood flow resistance (9, 10).

The purpose of this study is to answer the following questions. Is the length–tension curve of vascular smooth muscle shifted by changes in agonist concentration? Are concentration dependent shifts of the length–tension curve mediated by a membrane receptor? When agonist concentration is maintained constant, will changes in extracellular calcium cause shifts of the length–tension curve? Accordingly, length–tension curves have been obtained from dog anterior tibial artery rings at different bath concentrations of norepinephrine, potassium, and calcium.

Methods. *Preparation of vessels.* The dog anterior tibial artery was used in all experiments. A 5.0-cm segment was excised and placed in a dissection bath containing a physiological salt solution (PSS) at 37°C. It was bubbled with a mixture of 95% O_2 and 5% CO_2. The adventitia was dissected from the vessel and cuts were made perpendicular to the longitudinal axis of the vessel. The cuts were approximately 2.0 mm apart in the middle portion of the segment. Ring width is the distance between the two cuts. Two stainless steel wires (0.33-mm diam) were inserted through the lumen of the ring. Each wire formed a triangle with the corner that was opposite the side in the ring (base) attached to a supporting hook. The rings equilibrated in the bath for 1 hour at a 1.0-g preload. A continuous flow of fresh PSS was provided at 37°C. pH was maintained by bubbling with a 95% O_2 and 5% CO_2 gas mixture. The normal PSS had the following composition (m*M*): NaCl, 115.0; KCl, 5.0; NaH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; $CaCl_2$, 1.7; glucose, 11.0; and $CaNa_2$ EDTA, 0.026. The pO_2, pCO_2, and pH of the PSS from the specimen bath were measured on a Corning 165 blood gas analyzer. The pO_2 = 481 mm Hg, pCO_2 = 31 mm Hg, and pH = 7.36.

Experimental apparatus. Distance between the wires in the ring was adjusted by a micrometer. Force was measured by a Statham UC2 transducer. The apparatus has been described previously (11). The internal circumference, media thickness, and width of the ring were measured on-line with a noncontact electronic video caliper. The magnification factor was 72. A digital meter (precise to 10 μm) provided a readout of each distance. In other experiments we found that on-line measurement of media thickness did not differ significantly from light microscopy measurements of stained sections of arterial rings fixed in glutaraldehyde (11).

Length–tension experiments. Length is defined as the distance around the inside edge of the ring. It was computed as twice the distance from the lower edge of the upper wire in the lumen to the upper edge of the lower wire, plus the wire circumference and two wire diameters. Media thickness and width of the ring were measured at a point half the distance between the supporting wire. All measurements were made with the video caliper. The initial length for passive force (L_0) was determined first by shortening the ring until resting force was zero. Then the ring was slowly lengthened until passive force was first detected by the force transducer. The internal circumference of the ring at this position of the micrometer was measured as L_0. The ring was then stretched in 20% increments of L_0 (approximately 0.35 mm). At lengths greater than 240% L_0 the increment of stretch was 10% L_0. After each stretch the viscoelastic force was allowed to decrease to a steady state value (approximately 15 min). The steady state viscoelastic force was taken as the passive force at all lengths (see Data Analysis). The arterial ring was stimulated at selected increments of stretch. After each stimulation the ring was allowed to relax to the resting force that was present before stimulation. At short lengths, this occurred in less than 2 min. However, a minimum of 4 min was allowed before stretching to the next lengths. The relaxation time increased with length. At lengths greater than L_{max} the relaxation time was approximately 10 min. Length was increased until the additional force resulting from stimulation decreased in two successive contractions. The length for maximum active force (L_{max}) is defined as the ring circumference at which maximum force is first recorded. With potassium stimulation at lengths close to L_{max} the rings were stimulated at 20% increments of L_0. Because L_0 averaged from 1.70 to 1.8 mm then the accuracy of determining L_{max} was approx-

imately ±0.18 mm. L_{max} averaged from 5.2 to 6.4 mm. Thus, the accuracy, as a percentage of L_{max}, is ±2.8 to ±3.5%.

Two groups of dogs were used for the norepinephrine experiments. Two curves were obtained from each dog at the same concentration to determine the effect of repetition on the length–tension relationship. In one group, two length–tension curves were obtained with 10^{-5} *M* NE. In the second group, two length–tension curves were obtained with 10^{-6} *M* NE. ED_{50} at L_{max} for NE is approximately 10^{-6} *M* in the dog anterior tibial artery (12). Preliminary studies of norepinephrine length–tension curves with 10^{-5} *M* and 10^{-6} *M* from the same ring were presented in a FASEB symposium (13).

In a separate group of dogs length–tension curves were obtained with 90 and 30 mM potassium (K^+). The ED_{50} at L_{max} for K^+ is approximately 30 mM in the dog anterior tibial artery (12). In one group of dogs, length–tension curves with high concentration were obtained first and curves with low concentration were obtained second. In a second group the low concentration curve was obtained first and the high concentration curve obtained second. Different concentrations of K^+ (30 and 90 mM) was achieved by changes in K_2SO_4 which was substituted for KCl. For each concentration of K^+ the amount of NaCl was adjusted to maintain a constant osmotic pressure (12). Calcium length–tension experiments were performed in a separate group of dogs with 1.7 mM Ca^{2+} (normal PSS) and with 0.085 mM Ca^{2+}. The agonist in all calcium experiments was 10^{-5} *M* norepinephrine. When the low calcium experiments were performed the ring was bathed in low calcium solution through the entire time of each experiment. This includes the use of low calcium solution when norepinephrine was injected into the bath and when norepinephrine was washed out of the bath. When normal calcium experiments were performed the ring was bathed in normal calcium solution through the entire time of the experiment including injection of norepinephrine and washout of norepinephrine. An equal number of experiments were performed with normal calcium or low calcium for the first curve.

Data analysis. We have shown in other experiments (11) that no decrease in force of the resting anterior tibial artery occurs at L_{max} when normal PSS is replaced by a calcium-free PSS. We have performed additional experiments which show no difference in resting force in normal PSS when compared to force in calcium-free PSS over the entire range of lengths in this study. Accordingly, we define the additional force developed after stimulation as active force. Force in the resting ring was defined as passive force. Stress was computed as force divided by the cross-sectional area of the media at L_{max}. The initial length for active force (L_i) was determined by the intercept of a straight line connecting the responses at the two shortest lengths (L_0 and 140% L_0) with the length coordinate on the length–tension graph.

Experimental values of force, stress, and length are reported as group means ± SEM. The two-tailed Student's *t* test was used to determine statistically significant differences in the mean. The combined effect of length and concentration on active tension was determined by an analysis of variance (ANOVA). *P* values, variance ratio (*F*), and degrees of freedom (*df*) are given for the ANOVA tests.

Results. *The effect of reference length on length–tension curves from the same ring.* Length–tension curves using high and low concentrations of K^+ from a single ring are shown in Fig. 1. In Fig. 1A, length is presented in absolute units (mm). In Fig. 1B length is normalized to L_0, the initial resting length. It can be seen that the relative position of the two curves is the same for normalized length (Fig. 1B) and absolute length (Fig. 1A). If the length for maximum response L_{max} in a maximal activated ring (90 m*M* K^+) is used as the reference length then the normalized length would change to L/L_{max}. The curves with L_{max} as the reference length are shown in Fig. 1C. The curves in Fig. 1C have the same relative position as Figs. 1A and B. Hence, the choice of reference length for the vessel ring does not alter the relative position of the curves.

In mechanical studies of blood vessels only one reference length is used to determine the mechanical relationships for each specimen. The specific reference length varies from study to study and L_{max}, L_0, L_i, or any other well-defined length may be used depending on the purpose of the study. However, from Fig. 1A it can be seen that the determination of L_{max}

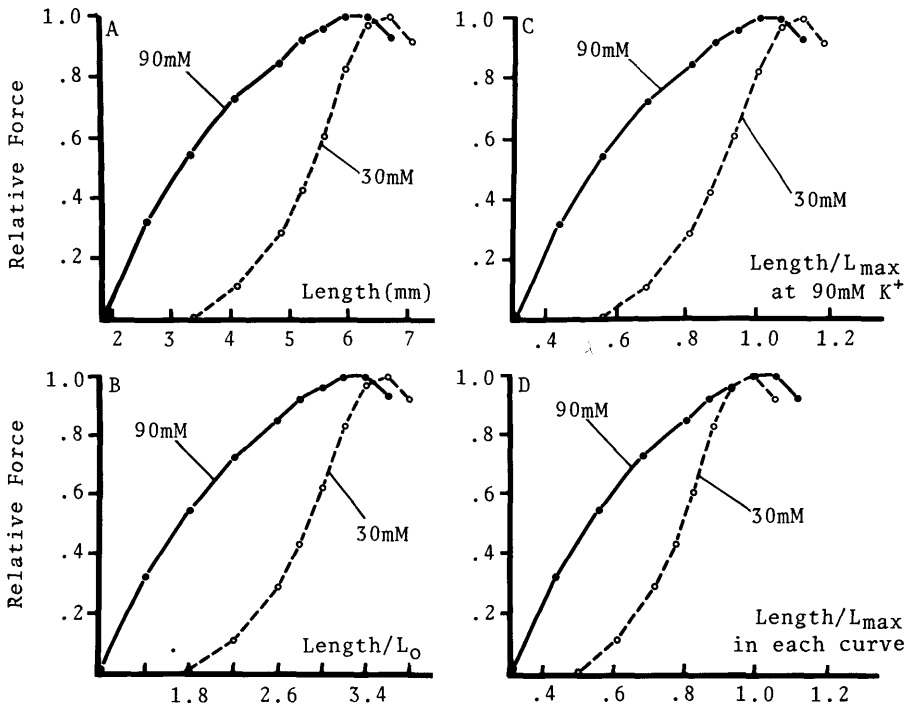

FIG. 1. Length–tension curves from a single arterial ring with potassium stimulation. (A) Length is given in absolute units. (B) Length is normalized to L_0 the initial resting length for the arterial ring. (C) Length is normalized to L_{max} the length for maximum force at maximal activation (90 m*M* K^+). (D) Length is normalized to the length for maximum response *for each curve.*

depends on the concentration of potassium. If the length–tension curves from 90 and 30 mM K^+ are normalized to their respective lengths for maximum response then the curves are shifted closer to each other. However, the curves in Fig. 1D still do not superimpose on one another. These figures show that when the same reference length is used for all curves from a specimen (Figs. 1B, C) any horizontal shift of the curve is the same as the shift found in a graph with absolute length (Fig. 1A). In contrast, when each curve from the same specimen is normalized to its own reference length (Fig. 1D) which may have different absolute values, the shift of the curve is not the same as when absolute length is used (Fig. 1A). Because the purpose of this paper is to detect horizontal shifts of the length–tension curve to different ranges of absolute length the same reference length must be used for both curves. Either L_0 or L_{max} are acceptable reference lengths. It is necessary to use one specific reference length in order to account for variations in the size of the animals. We have chosen to use L_0 as a reference length because L_{max} requires a series of length elongation and stimulations to determine its value before two curves can be obtained.

Indices of the relative position of two curves include L_i, L_{max}, and the effective length for one-half the maximum response in a length–tension curve or EL_{50}. EL_{50} is similar to ED_{50} in dose–response experiments. For the 90-m*M* K^+ curve in Fig. 1B a line connecting the response at 1.40 L/L_0 to the response at 1.8 L/L_0 passes through the relative response of 0.5. Then the EL_{50} for 90 m*M* K^+ is 1.70 L/L_0 and for 30 m*M* it is 2.87 L/L_0. The absolute value of EL_{50} (in mm) with 90 m*M* potassium is significantly smaller than it is with 30 m*M* potassium (Table I). In other words the arterial ring is more "sensitive" to length when the potassium concentration is high than when it is low.

Length–tension experiments with potassium stimulation. The results from length–tension

TABLE I. COMPARISON OF LENGTH-TENSION CURVES OBTAINED WITH 90 m*M* POTASSIUM TO CURVES OBTAINED WITH 30 m*M* POTASSIUM

	90 m*M*	30 m*M*
L_0	1.79 ± 0.03	1.79 ± 0.03
L_i	1.80 ± 0.08	3.0 ± 0.16*
EL_{50}	3.18 ± 0.10	4.39 ± 0.30*
L_{max}	5.48 ± 0.10	5.99 ± 0.16*
L/L_0 at L_{max}	3.07 ± 0.04	3.35 ± 0.08*
Active force at L_{max}	23.7 ± 1.4	11.3 ± 1.6*
Resting force at L_{max}	3.5 ± 0.4	6.1 ± 0.8*
Active stress at L_{max}	290 ± 19	141 ± 18*
Resting stress at L_{max}	42 ± 6	75 ± 9*

Note. $N = 12$; $df = 22$. *Significant difference symbols. Units of length, mm; force, g; stress, 10^4 dynes/cm^2.

experiments with potassium stimulation are shown in Fig. 2 and in Table 1. Length is normalized to L_0, the initial length for resting force, and force is normalized to the maximum active force in a length-tension experiment. If the length-tension curve is independent of K^+ concentration then the normalized curves should be superimposed on one another. However, the two curves in Fig. 1 are not superimposable. The normalized force is significantly different at each length from 100 to 320% L_0. The length for maximum active force (L_{max}) with 30 m*M* K^+ is significantly longer than with 90 m*M* K^+. The longer initial length for an active response (L_i) with 30 m*M* K^+ is highly significant. As expected the active response at L_{max} is significantly higher with 90 m*M* K^+ than with 30 m*M* K^+. Corresponding to the longer L_{max} with 30 m*M* K^+ is a significantly larger resting force (or stress) at L_{max}.

In potassium length-tension experiments one curve was obtained with 90 m*M* K^+ and one curve was obtained with 30 m*M* K^+ from each ring. An equal number of experiments were performed with 90 or 30 mM K^+ as the first concentration and the combined data were given in Table I. In addition, the results from the two groups have been analyzed separately. When the 90-m*M* K^+ curve is obtained first the two curves are more widely separated than when the 30-m*M* K^+ curve is

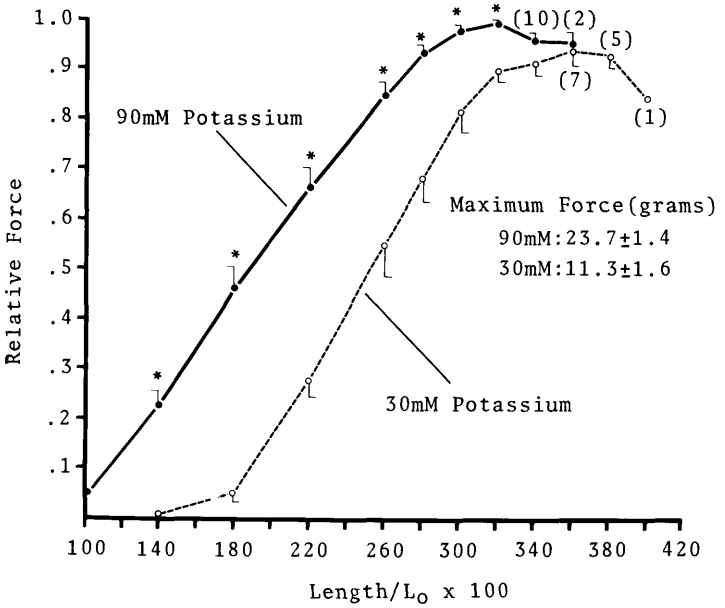

FIG. 2. Normalized length-tension curves obtained with 90 and with 30 m*M* potassium stimulation. Relative force is the active force at each length divided by the maximum active force in a curve. The difference between the two curves is highly significant (indicated by * for $P < 0.001$) from 100% L_0 to 320% L_0. See Table I for a summary of other characteristics of the length-tension curves. Each symbol represents the mean value of relative force from 12 measurements except at long lengths where the number of measurements is given in parentheses. ANOVA analysis for length is $P < 0.01$ ($F(7,176) = 217$) and for concentration is $P < 0.01$ ($F(1,176) = 177$).

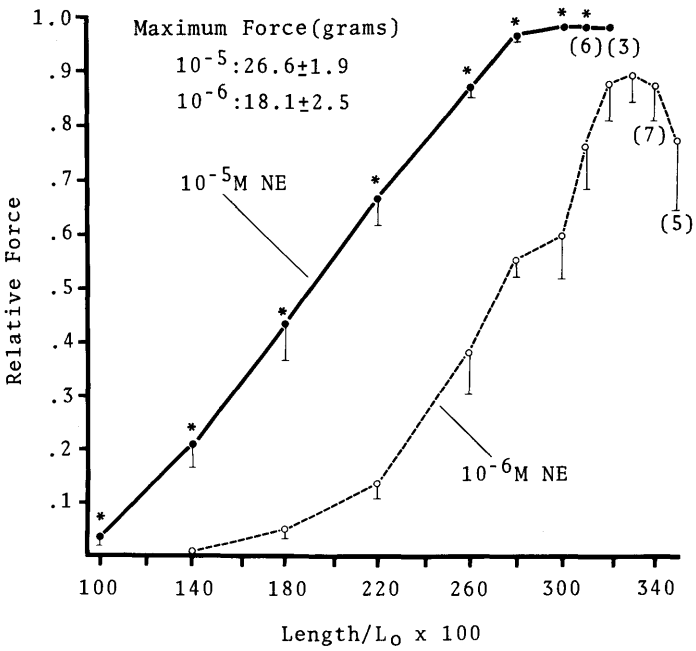

FIG. 3. Normalized length–tension curves obtained with 10^{-5} *M* norepinephrine and with 10^{-6} *M* norepinephrine stimulation. The difference between the curves is significant ($P < 0.001$) from 100% L_0 to 310% L_0. See Table II for other characteristics of the curves. Each symbol represents a mean value from eight measurements of active force except where the number is given in parentheses. ANOVA analysis for length is $P < 0.01$ ($F(6,98) = 77$) and for concentration is $P < 0.01$ ($F(1,98) = 170$).

obtained first. However, the 30-m*M* K^+ curve is always to the right of the 90-m*M* K^+ curve. Thus, the order of obtaining length–tension curves has a small effect but does not change the results of the experiments.

Length–tension experiments with norepinephrine stimulation. The results from norepinephrine length–tension experiments are shown in Fig. 3 and Table II. For each dose of norepinephrine there were four dogs, one arterial ring from each dog, and two length–tension curves were done for each ring. The two length–tension curves were obtained from the same ring with the same concentration of norepinephrine (10^{-5} or 10^{-6} *M*). Thus, the mean values in Fig. 3 and Table II represent eight measurements of active force at each length. For 14 *df* the difference in relative force between 10^{-5} *M* and 10^{-6} *M* is significant at each length over the entire range of the ascending curve (i.e., from 100 to 310% L_0). L_{max}, EL_{50}, and L_i are significantly less for 10^{-5} *M* NE than for 10^{-6} *M* NE. Corresponding to the shorter L_{max} for 10^{-5} *M* NE is the lower resting force at L_{max}. As expected the active force is higher for 10^{-5} *M* NE than for 10^{-6} *M* NE. The results from the first length–tension curve obtained with 10^{-5} *M* norepinephrine in four dogs have been compared to the first length–tension curve obtained with 10^{-6} *M* norepinephrine in the other group of dogs.

TABLE II. COMPARISON OF NOREPINEPHRINE LENGTH–TENSION EXPERIMENTS OBTAINED WITH 10^{-5} *M* TO EXPERIMENTS WITH 10^{-6} *M* ($n = 8$)

	10^{-5} *M*	10^{-6} *M*
L_0	1.83 ± 0.10	1.70 ± 0.03
L_i	2.08 ± 0.17	2.73 ± 0.14*
EL_{50}	3.42 ± 0.19	4.79 ± 0.18*
L_{max}	5.26 ± 0.14	5.65 ± 0.07*
L/L_0 at L_{max}	2.88 ± 0.03	3.31 ± 0.05*
Active force at L_{max}	26.6 ± 1.9	18.1 ± 2.5*
Resting force at L_{max}	4.2 ± 0.6	6.8 ± 0.8*
Active stress at L_{max}	389 ± 33	258 ± 40*
Resting stress at L_{max}	67 ± 8	94 ± 12

Note. $N = 8$; $df = 14$. See Table 1 for units and significant difference symbols.

In this case $N = 4$ and the $df = 6$. L_i, EL_{50}, and L_{max} were significantly longer for 10^{-6} M than for 10^{-5} M norepinephrine. Similarly, a comparison of the second curve obtained with 10^{-5} M norepinephrine to the second curve obtained with 10^{-6} M norepinephrine in the two groups of four dogs showed L_i, EL_{50}, andL_{max} to be significantly longer for 10^{-6} M. A comparison of the first curve obtained with 10^{-5} M to the second curve obtained with 10^{-5} M in the same dogs showed no significant difference between L_i, EL_{50}, and L_{max}. The same result was found when the first curve obtained with 10^{-6} M was compared to the second curve obtained with 10^{-6} M in the other group of dogs. Thus, the effect of repeating norepinephrine length–tension experiments is insignificant compared to the effect of concentration.

The effect of external calcium concentration on the length–tension relationship. The results from length–tension experiments for the effect of external calcium concentration are shown in Fig. 4 and Table III. As expected the maximum active force and the corresponding stress is significantly lower with low calcium (0.085 mM) than with normal calcium (1.7 mM). However, none of the parameters associated with shifts in the length–tension curve (L_i, EL_{50}, or L_{max}) are significantly different. This is confirmed by the length–tension curves in Fig. 4 where no significant difference between the relative forces at any length are found.

The calcium length–tension experiments yielded a curve with normal external calcium concentration and 10^{-5} M NE and a curve with low external calcium concentration and 10^{-5} M NE from the same arterial ring. An equal number of experiments were performed with normal Ca^{2+} or low Ca^{2+} as the first concentration. The results have been separated into the group of vessels where the curve with normal calcium curve was obtained first and the group of vessels where the curve with low calcium was obtained first. All of the reference lengths (L_i, EL_{50}, and L_{max}) are not significantly different regardless of which concentration of calcium was used first. The general

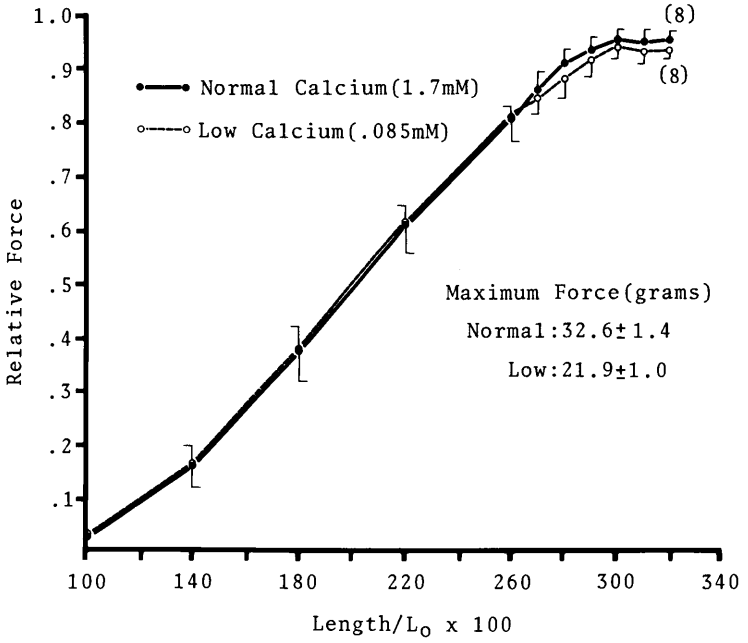

FIG. 4. Normalized length–tension curves obtained with 10^{-5} M norepinephrine and 1.7 mM calcium and with 10^{-5} M norepinephrine and 0.85 mM calcium. There is no significant difference between the two curves. See Table III for a summary of other characteristics of the curves. Each symbol represents the mean of 10 measurements except where a number is given in parentheses. ANOVA analysis for length is $P < 0.01$ ($F(9,180) = 210$) and for concentration there is no significant difference ($F(1,180) = 0.34$).

TABLE III. COMPARISON OF LENGTH–TENSION EXPERIMENTS OBTAINED WITH NORMAL CALCIUM TO EXPERIMENTS OBTAINED WITH LOW CALCIUM

	Normal (1.7 m*M*)	Low (0.085 m*M*)
L_0	1.81 ± 0.06	1.81 ± 0.06
L_i	1.89 ± 0.12	1.95 ± 0.14
EL_{50}	3.58 ± 0.16	3.67 ± 0.16
L_{max}	5.48 ± 0.14	5.68 ± 0.15
L/L_0 at L_{max}	3.04 ± 0.06	3.15 ± 0.07
Active force at L_{max}	32.6 ± 1.4	21.9 ± 1.0*
Resting force at L_{max}	4.5 ± 0.4	6.5 ± 1.0
Active stress at L_{max}	342 ± 18	249 ± 19*
Resting stress at L_{max}	50 ± 7	72 ± 9

Note. $N = 10$, $df = 18$. See Table 1 for units.

trend for L_i, EL_{50}, and L_{max} is to be longer for the length–tension curve that is obtained second when compared to the curve that is obtained first regardless of the concentration of calcium. This illustrates the effect of repeated stretching on obtaining length–tension curves.

To determine if a change in calcium concentration alters the resting stress in the dog anterior tibial artery length–resting stress curves were obtained from a separate group of dogs. The arterial ring was stretched in increments of L_0. At each length the resting stress was recorded first in normal calcium solution (1.7 m*M*), then in a 0.085-m*M* Ca^{2+} solution, and finally in a calcium-free solution. As shown in Fig. 5 there is no change in resting stress at any length for the three concentrations of calcium.

Discussion. It is well known that the magnitude of contractile force in vascular smooth muscle depends on length and on the concentration of agonist. In previous work we have shown that the dose–response relationship between concentration and contractile force depends on muscle length (12, 8). To determine a shift of the dose–response relationship irrespective of change in magnitude, the active force at each concentration was normalized to the maximum force. In this paper, active force is normalized to maximum active force in order to detect horizontal shifts of the length–tension relationship. Our experiments show that the length–tension relationship in vascular smooth muscle is shifted by the concentration of agonist. This has been demonstrated with potassium stimulation and with norepinephrine stimulation. However, shifts of the rela-

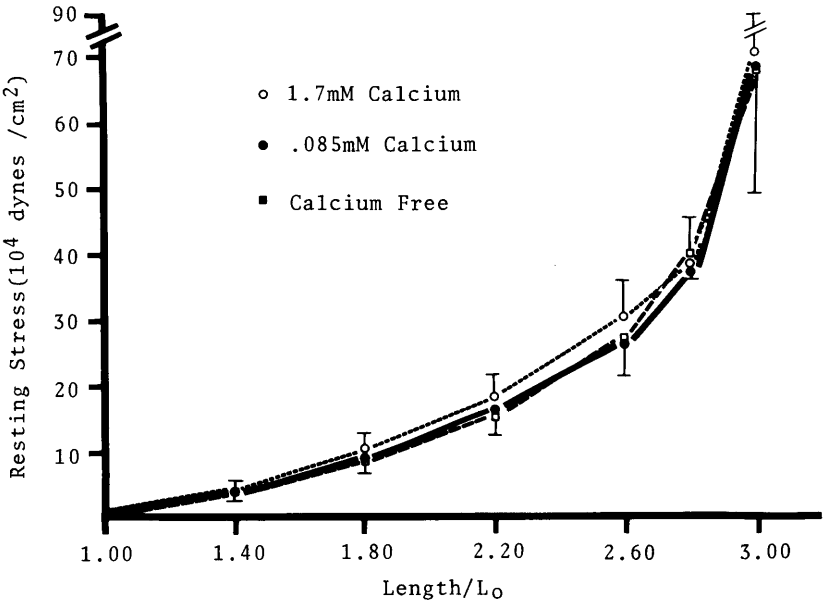

FIG. 5. The effect of calcium concentration on the length–resting stress relationship. At each length the ring was exposed to a PSS containing 1.7 m*M* Ca^{2+}, then to a PSS with .085 m*M* Ca^{2+}, and then to calcium-free solution.

tionship were not found when the bath concentration of calcium is changed and norepinephrine is the agonist. As expected, we found the absolute response (in grams) at each length is significantly different with changes in the concentration of potassium, norepinephrine, or calcium. We conclude that the length–tension relationship of the anterior tibial artery is shifted horizontally along the length axis according to the degree of activation by a chemical agonist. This effect is not dependent on the membrane receptor for norepinephrine nor on the amount of extracellular calcium.

We have shown that the choice of reference length does not alter the relative position of length–tension curves from the same arterial ring. The reference length chosen for this study is the initial length for resting force L_0. The determination of L_0 in the anterior tibial is not affected by the presence of any intrinsic tone. If tone were present the true L_0 would not be determined. However, once a specific reference length is determined for a particular ring with tone then according to our results the relative position of length–tension curves will not be affected by the choice of reference length. Of course the magnitude of the response in length–tension curves will be affected by tone. If the degree of tone is highly variable from one ring to another this will increase the variability of the reference length which could increase the standard errors of the responses at specific increments of L_0.

In our earlier papers (12, 8) on a length-dependent dose–response relationship and in this paper the same three parameters are measured: active force (or tension), agonist concentration (or dose), and length. Since the relationship between agonist concentration and active force was shown to be length dependent then it follows that the relationship between length and active force must depend on agonist concentration. The results from our earlier dose–response experiments indicated that we could expect this result from length–tension experiments at different concentrations (see Discussion in Ref. (8)). We have verified that prediction in this paper. Because of these results, we suggest that the dependence on the dose–response relationship on length and the dependence of the length–tension relationship on agonist concentration are physical manifestations of the same biological mechanism.

In agreement with our result of no difference in length–tension relationships at different calcium concentrations, Herlihy and Berardo (14) found no change in the ED_{50} of calcium dose–response relationships at different preloads. They used rat aortic strips in high potassium.

Shifts of the length–tension relation with various activation procedures and possible mechanisms have been widely investigated in skeletal muscle and heart muscle. These changes were first described by Rudel and Taylor in 1971 using frog skeletal muscle (15). In the studies that followed, it has been generally agreed that the depression of the length–tension curve at short lengths in intact skeletal and heart muscle reflects an alteration of the activation process due to the change in length (1, 3–6). Mechanisms not associated with excitation–contraction coupling such as extrinsic restoring forces are also possible but most have been discounted in favor of the role for activation processes of the contractile system (1).

Although studies on striated muscle suggest that length-dependent activation is related to the role of calcium, certain characteristics of the excitation–contraction coupling process are different in heart muscle, skeletal muscle, and smooth muscle (see Discussion in Ref. (6)). As a result, the magnitude of the contractile response in skeletal muscle is insensitive to changes in external calcium, whereas in heart muscle and smooth muscle it is very sensitive. In addition to changes in the magnitude of active force, horizontal shifts of the length–tension relationship is sensitive to external calcium (from 1.125 to 4.5 m*M*) in heart muscle (4) whereas in smooth muscle (our study) shifts of the length–tension relationship are insensitive to external calcium (from 1.7 to 0.085 m*M*).

The results from our calcium experiments mean that an increase in extracellular calcium increases the magnitude of contractile force but the change in force is proportionally the same at all lengths. Winquist and Bevan (16) have shown that tone in rabbit facial vein depends on stretch and on the concentration of extracellular calcium. They proposed that the results could be due to a stretch-induced change of calcium permeability of smooth muscle cells, altered overlap of contractile filaments, increased affinity of calcium regula-

tory proteins, or other mechanisms. In their paper the length–tension (instinsic tone) curves at different concentrations of external calcium appear to have the same L_i and L_{max} (i.e., the curves would superimpose if the responses were normalized to the maximum). Thus, length (or stretch) does not appear to alter the effect of extracellular calcium in rabbit facial vein. These results do not exclude a role for calcium in the shift of length–tension curve when agonist concentration is altered. For example, in the anterior tibial artery the release of intracellular calcium by norepinephrine could be length dependent. Further studies are required on the exact role of calcium.

There are other possibilities for a mechanism of concentration-dependent length–tension curves in vascular smooth muscle. These include an inhibition of the release of an endothelial-derived relaxation factor (EDRF) from the endothelium with the subsequent facilitation of the contractile response at longer lengths. For the same amount of EDRF the sensitivity to a low dose will be depressed proportionally more than a high dose at a given length. Nilsson and Sjoblom (17) proposed that an increase in sensitivity might have its origin in the contractile machinery of the muscle cell. At short lengths small contractions would be absorbed by the series element and a measurable force would be generated only with stronger activation. This mechanism would apply equally to cell agonists. Based on stereological analysis of dense body distribution, Walmsley (18) has suggested that a change in orientation of intracellular contractile units as a function of cell length may play role in length-dependent sensitivity.

Regardless of the mechanism for length-dependent activation it is now well accepted that length and the contractile (or inotropic) state in heart muscle are not independent regulators of tension. Indeed, length is thought to affect activation in a manner similar to inotropic agents (4). We suggest a similar point of view for length and contractility in vascular smooth muscle. In agreement with studies on heart muscle (4) and skeletal muscle (20), our results show that the length for maximum response in vascular smooth muscle is shorter as the magnitude of activation is increased (i.e., increased concentrations of norepinephrine and potassium). Horizontal shifts in the length–tension curve of rat cerebral microvessels can be observed in the studies of Halpern *et al.*, (19). In Fig. 6 of their paper the length–tension curve for the myogenic response in normal calcium PSS was clearly to the right of the curve with high potassium PSS. The myogenic response in normal calcium PSS at each length was lower in magnitude than the maximum response to high potassium. Further support for length-dependent activation in smooth muscle comes from Arner and Hellstrand (7) who found a lower relative force at lengths below L_{max} in intact segments of rat portal vein than in skinned preparations. Their results are similar to the results for skinned heart muscle (3) and skinned skeletal muscle (2).

One distinction should be made concerning the various studies of length-dependent activation. Experiments with skinned preparations show that their response is greater than intact muscle at short lengths and help to elucidate the mechanism of length-dependent activation (2, 3, 7). Studies on intact muscle show that change in activation levels produce shifts in the length–tension relationship. Hence, there exists an intrinsic depressive effect on contractile force due to a suppression of the activation process when intact muscle is shortened. Further, the degree of this intrinsic depressive effect is modulated according to the degree of activation by an external chemical or electrical stimulus. This suggests that shifts of the length–tension relationship by changes in agonist concentration alters the control of blood flow resistance by blood vessels. For example, the magnitude of vasoconstriction is a linear function of resistance prior to stimulation in the vasculature of the dog hind limb (10). Because the initial resistance imposes different degrees of stretch on the smooth muscle its capacity to respond is altered through the length–tension relationship. Studies by Gore (9) show that single vessels do respond to a constant dose of norepinephrine in proportion to their initial wall stress. In view of our results the *in vivo* effect of concentration changes is to reset the length–tension relationship to a different range of arterial diameters. Shifts of the length–tension relationship through change in agonist concentration have been indicated by Speden (21) with perfused cylindrical segments of the rabbit ear

artery. In his experiments the effect of varying adrenaline concentration was to vary the radius at which active tension was first developed. This corresponds to our result of a significant increase in the initial muscle length that is required for an active response (L_i) when agonist concentration is decreased.

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