THE UNDAMPED AND DAMPED SERIES ELASTIC COMPONENTS OF A VASCULAR SMOOTH MUSCLE

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ABSTRACT Small arterial resistance vessels (internal diameter about 175 μm) have been mounted on a myograph that enabled their wall tension, T, and internal circumference, L, to be measured and controlled with a time resolution of about 4 ms. Maximally activated vessels were subjected to isometric releases (step changes in L) and isotonic releases (step changes in T) of varying extents and at two different temperatures (27°C and 37°C). The recovery from an isometric release was monotonic and did not include the two phases seen in skeletal muscle. The isotonic release response did, however, contain a velocity transient lasting about 150 ms: the velocity immediately after the release was about six times the steady shortening velocity. The form of both the isometric and isotonic release responses and their dependence on the extent of release can be explained in terms of a modified Hill model in which the "series elastic component" (SEC) is replaced by the series combination of an undamped-SEC (that is, an undamped elastic element) and a damped-SEC (a Voigt element). Although the initial response to both types of release was independent of temperature, all stages of subsequent responses were temperature dependent, with Q_{10}'s in the range 1.5–2.0. The results suggest that the responses to isotonic and isometric releases may in part be due to active processes.

INTRODUCTION

The mechanical properties and location of the series elastic component (SEC) of smooth muscle have recently been the subject of a number of investigations (Stephens and Kromer, 1971; Herlihy and Murphy, 1974; Mulvany and Halpern, 1976; Alexander, 1976; Fay, 1977; Cox, 1977; Dobrin and Canfield, 1977; Meiss, 1978). Although there is general agreement that this component is less stiff than the corresponding component of skeletal muscle, there is disagreement concerning its location. Much of this disagreement is, however, one of semantics. The term "series elastic component" was first introduced by Hill (1938). Hill showed that the mechanical properties of whole activated skeletal muscle could be described approximately by an undamped spring (the SEC) in series with a contractile component (CC) whose rate of shortening was determined solely by the load imposed on it according to "Hill's equation" (Fig. 1 *a*). Hill did not himself ascribe a location for the SEC, but the later discovery of cross-bridges as the fundamental force generating units in muscle encouraged others to treat Hill's concept as a morphological model where the CC represented the cross-bridges and the SEC represented the passive structures in series with them. Refined mechanical measurements on skeletal muscle have shown however (Jewell and Wilkie, 1958; Civan and Podolsky, 1966; Hill, 1970; Huxley and Simmons, 1971; Bressler and Clinch, 1974; Abbott and Steiger, 1977) that Hill's (1938) model does not, in fact, provide a complete description of activated muscle. Its behavior is better (but still not perfectly) described by considering the SEC as itself a series combination of undamped and damped elastic

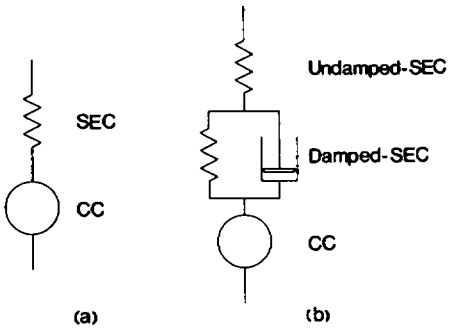

FIGURE 1 (*a*) Diagram of Hill's (1938) model for describing the mechanical properties of activated muscle. The SEC is a spring while the CC obeys Hill's equation, $(P + a) \cdot (v + b) = \text{constant}$, where P is the active tension, v is the shortening velocity, and a and b are constants. (*b*) Modified form of Hill's model in which SEC of a is replaced by the series combination of an undamped-SEC and a damped-SEC. The undamped-SEC is a spring, the damped-SEC is a Voigt element, i.e., the parallel combination of a spring and a dashpot. The springs are nonlinear. The CC has the same characteristics as in *a*.

components (Ford et al., 1977), as shown in Fig. 1 *b*. The morphological equivalent of these components is still not established, but it seems likely some of the undamped series elasticity of skeletal muscle lies within the cross-bridges themselves, while the damped elasticity may also be a property of the cross-bridges. Therefore, taking Hill's model to indicate a morphological division between a passive SEC and an active CC can lead to confusion.

In this paper the term SEC is used in its original manner as a mathematical description of components that must be placed in series with a CC (obeying Hill's equation) to mimic the behavior of activated muscle. As we shall see, the experimental results indicate that on this basis the SEC in smooth muscle can also be adequately described by dividing the SEC into an undamped-SEC and a damped-SEC connected in series.

In skeletal muscle the properties of the SEC have been investigated by subjecting single fibers to rapid changes in length "isometric releases," (e.g., Huxley and Simmons, 1971) or to rapid changes in load "isotonic releases," (Civan and Podolsky, 1966). The former method is technically easier, but suffers from the disadvantage that the presence of a damped elasticity can be masked by undamped elastic elements, because these will change length at a varying rate during the redevelopment of tension. These difficulties are not found in isotonic releases, for here the undamped series elastic elements maintain the same length after the change in load. In the current investigation both methods have been used and the resulting responses studied at 37°C and 27°C, with the intention of differentiating between those responses arising from passive structures and those arising from active structures or processes.

The preparation used is a small arterial resistance vessel with lumen diameter of about 150 μm (Mulvany and Halpern, 1976). This preparation has the advantage that it is small enough for the smooth muscle cells within the walls to be directly visualized, thus enabling direct correlation to be made between the mechanical properties of the preparation and the properties of the cells. Moreover, its small size simplifies the apparatus that is necessary to make dynamic measurements with a higher time resolution (~4 ms) than has been generally possible with other smooth muscle preparations.

METHODS

Arterial resistance vessel segments (length ~0.8 mm, lumen ~175 μm) taken from the mesenteric bed of 3–4-mo-old Wistar rats, were threaded on to two stainless steel wires that were attached, respectively, to a force transducer and a piezoelectric displacement device ("pusher"), as shown in Fig. 2. The basic details of this myograph and the method of dissection and mounting have been described elsewhere (Mulvany and Halpern, 1976, 1977). The arrangement enables the wall tension, T, and internal circumference, L, of the vessel to be continuously monitored and controlled. The whole myograph is mounted on a microscope stage equipped with Nomarski interference contrast optics. In the current investigation the overall stability of the myograph has been improved so that dynamic measurements can be made without the need for signal averaging, as was the case in the previous work.

The solutions used had the following compositions (in mM): Physiological salt solution (PSS): NaCl, 119; $NaHCO_3$, 25.0; KCl, 4.7; KH_2PO_4, 1.18; $MgSO_4$, 1.17; $CaCl_2$, 1.6; ethylenediaminetetraacetic acid (EDTA), 0.026; glucose, 5.5. Activating solution: as for PSS but with an equimolar replacement of NaCl with KCl, a total of 2.5 mM $CaCl_2$ and the addition of 40 μM noradrenaline (Hoechst). Ca-free PSS: as for PSS but without $CaCl_2$ and with 1 mM ethyleneglycol-*bis* (β-aminoethyl ether)-N,N′-tetraacetic acid (EGTA). The solutions were aerated with 95%/5% O_2/CO_2 and adjusted to pH 7.4. Solutions were held at 37°C, except where otherwise indicated. Vessels were found to relax fully in PSS (Mulvany and Halpern, 1977) and there was never any sign of spontaneous activity. Activating solution produced a maximal response (Mulvany et al., 1978).

After about 30 min equilibration in PSS, vessels were set, for reasons given below, to an internal circumference $L_1 = 0.9\ L_0$, where L_0 is the internal circumference for which maximum wall tension is developed upon activation (Mulvany and Halpern, 1977). Here the thicknesses of the wall (~24 μm) and media (~13 μm) of the mounted vessels were measured with the microscope at 500 power, as

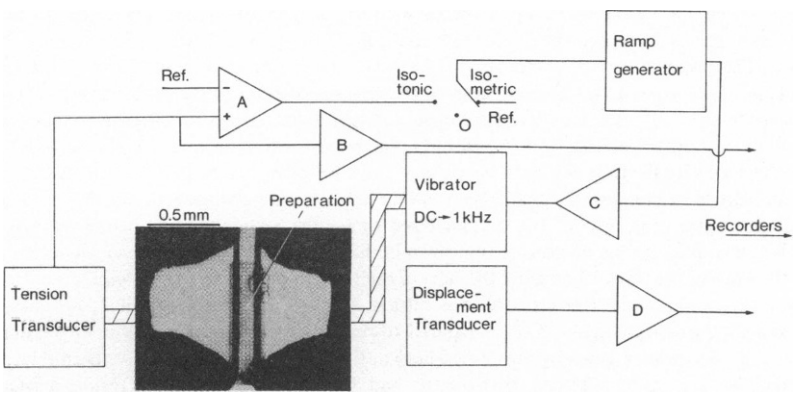

FIGURE 2 Schematic of myograph used in this investigation. The arterial segment (shown here, arrowed, in the low-power micrograph) was threaded on two wires attached, respectively, to a force transducer (DSC6, Kistler Morse Corp., Bellevue, Wash.) and a 15-μm range piezoelectric vibrator ("pusher" PZ-40, Burleigh Instruments Inc., Fishers, N.Y.). The pusher was mounted on a micrometer stage for larger movements. The displacement of the pusher arm was monitored by an eddy-current transducer (2300.5 SU, Kaman Sciences Corp., Colorado Springs, Color.) the output of which was filtered by an active low-pass filter, D, (−12 dB per octave above 300 Hz [Burr-Brown, 1966]). This displacement was determined by the output of a 1000 V, 100-gain operational amplifier, *C*, which was fed by a ramp generator. The ramp generator. The ramp generator input was normally 0V (switch at "O"), but for isometric or isotonic releases the input was changed, respectively, to a preset reference voltage or to the output of a differential feedback amplifier, *A*, as described in the text. The outputs of the force transducer amplifier, *B*, and of *D* were each recorded simultaneously by an oscillograph (7420A, Hewlett Packard, Co., Palo Alto, Calif.) and a storage oscilloscope (5103N/D11, Tektronix, Inc., Beaverton, Oreg.).

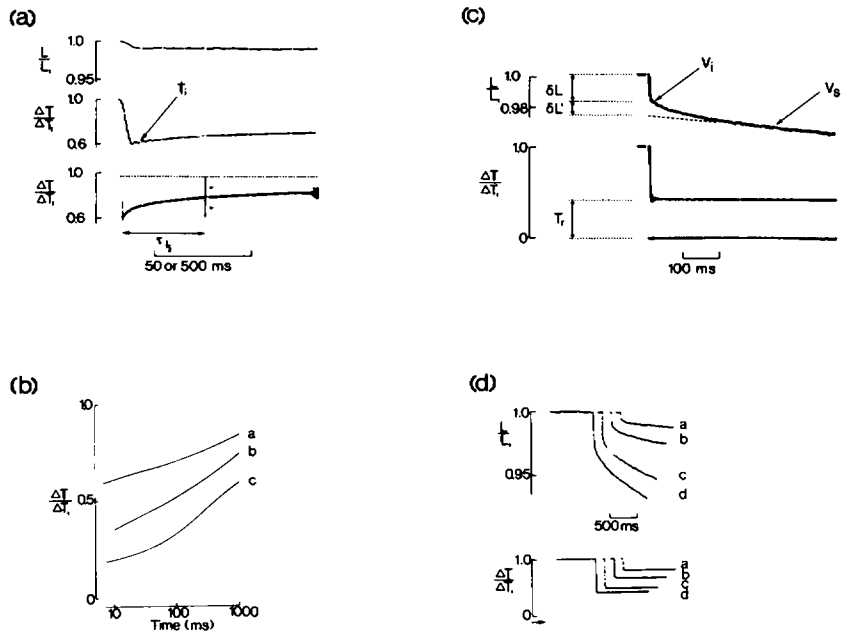

FIGURE 3 Isometric and isotonic release responses of activated vessels at 37°C. L is internal circumference, ΔT is active wall tension (i.e., wall tension in excess of resting wall tension before activation). (*a*) Isometric release recorded with oscilloscope by using two different time bases: bar is 50 ms for upper two traces and 500 ms for bottom trace. Top trace shows imposed internal circumference change, lower two traces the wall tension response. The dashed line above the bottom trace shows the wall tension 10 s after the release. The response was characterized by measuring (*i*) the initial rate of rise of wall tension, $\dot{T}_i$, from the slope of the trace 15 ms after the start of the release, as shown in the center trace, (*ii*) the active half response time, $\tau_{1/2}$, i.e., the time taken to recover halfway to the tension 10 s after the release, as shown in the bottom trace. (*b*) Wall-tension responses to isometric releases of different extents recorded as in *a*, but here replotted with the time axis on a logarithmic scale. Releases were *a*, 0.01 L_1; *b*, 0.02 L_1; *c*, 0.03 L_1. (*c*) Isotonic release recorded with oscilloscope showing imposed load change (center trace) and resulting displacement change (top trace). The bottom trace shows the resting tension before activation. The response was characterized by measuring (as shown in the top trace) (*i*) the initial velocity of shortening, V_i, from the slope of the trace 15 ms after the start of the release, (*ii*) the steady velocity of shortening, V_s, from the slope of the trace 300 ms after the release, (*iii*) the initial change in L, δL, and (*iv*) by construction the further change in L, $\delta L'$, required to reach the point on the back extrapolation of the steady velocity shortening (shown by the dashed line) at time 0. These quantities have been related to the isotonic load, T_r, that is, the difference between the load during the release and the resting tension before activation, as indicated on the lower traces. (*d*) Internal circumference responses to isotonic releases of different extents recorded with an oscillograph. The releases were to $T_r = a$, 0.80 ΔT_1; *b*, 0.67 ΔT_1; *c*, 0.43 ΔT_1; *d*, 0.40 ΔT_1. L_1/π was *a,b*: 130 μm; *c,d*: 197 μm. ΔT_1 was *a,b*: 2.56 mN/mm; *c,d*: 4.50 mN/mm.

described elsewhere (Mulvany et al., 1978). Vessels were then activated at L_1 for about 4 min every 15 min by changing the circulating solution to activating solution. Approximately 3 min after activation the vessels were subjected either to an "isometric" or an "isotonic" release (see below), which resulted in the internal circumference decreasing by up to 0.1 L_1. The vessels were subsequently relaxed by changing the solution back to PSS. When the vessels were fully relaxed (~5 min later) they were stretched back to L_1. In other words, vessels were only stretched when relaxed; under these conditions the response to activating solution remained constant (within ± 5%) throughout experiments, each of which lasted about 6 h.

Isometric releases were effected by switching the input to the ramp generator from "0 V" to a preset voltage level (Fig. 2). This resulted in the pusher moving in by the required amount over a period of ~6 ms (the period was deliberately extended to avoid setting up oscillations in the tension transducer). Isotonic releases were effected by switching the input to the ramp generator from "0 V" to the output of a feedback amplifier. This output was the difference between the (amplified) output of the tension transducer and a preset voltage level. Thus, switching to the isotonic mode caused the pusher to move in to reduce the tension to, and then maintain the tension at, the required level. The stability of the servoloop was maintained by feeding the derivative of the tension signal into the input of the feedback amplifier. Because of the nonlinearity of the vessel, the gain of the feedback amplifier had to be adjusted for each size of isotonic release (see Ford et al., 1977) to obtain optimum damping. With correct adjustment, the release was complete within about 6 ms (Fig. 3 *c*).

The overall frequency response of the myograph was about 250 Hz. The total compliance of the myograph, measured from the change in spacing of the mounting wires at their midpoint after activation, was < 0.5 μm/mN and no correction has been made for this.

The experiments described in this paper have been performed at two temperatures (37°C and 27°C), and the differences between the values of the parameters measured at each temperature have been tested for significance by using the 2-tail Student t test. The ratio of the parameters determined at the two temperatures—the Q_{10}—was also calculated. The mean values and SE's of the parameters and their Q_{10}'s are given in the Tables. In the text only mean values are presented.

The model calculations described in Fig. 8 were performed on the Department's Prime 300 computer (Prime Computers Inc., Framingham, Mass.). In some experiments the computer was also used on-line to acquire the data, making digital measurements at 2 ms intervals, and from these calculating the response parameters.

The method by which the preparation is mounted means that the terminology for describing its mechanical characteristics differs from that used for skeletal muscle or smooth muscle-strip experiments. The smooth muscle cells in the preparation are oriented circumferentially, so that, as regards these, the "length" of the preparation is the internal circumference. Changes in spacing between the mounting wires are taken to be distributed equally over the whole internal circumference, so that a given change in spacing produces an alteration in internal circumference equal to twice this change. Similarly, because the segment length is the arbitrary result of the dissection process, the "force" in the preparation is best expressed as a wall tension, that is, measured force divided by twice the segment length. The nomenclature used for these and related terms is as follows: L, internal circumference; L_1, normalized internal circumference as described above; L_1/π, "effective diameter" at L_1, i.e., vessel internal diameter if cross-section were circular; δL, initial change in L in an isometric or isotonic release (equal to twice movement measured by displacement transducer); V_i, V_s, initial and steady velocities of shortening (rates of decrease of internal circumference), respectively, in an isotonic release; T, wall tension (equal to force measured by force transducer divided by twice segment length); ΔT_1, active wall tension (wall tension of vessel when activated at L_1 by activating solution in excess of the wall tension of the relaxed vessel before activation); and $\Delta\sigma_1$, active media stress at L_1 (equal to active wall tension divided by media thickness).

RESULTS

Isometric and Isotonic Response Characteristics

Fig. 3 shows typical records of the two types of test to which vessels have been subjected: isometric and isotonic releases, as described in Methods. The wall-tension response to an isometric release (Fig. 3 *a*) was a drop in tension simultaneous with the decrease in internal circumference, followed by a tension recovery at a rate that decreased monotonically with time. For small releases (< 0.01 L_1) full recovery was obtained within ~10 s, but for larger releases the recovery took up to 1 min. In neither case could the time-course of the recovery be

expressed by a simple exponential equation. Their time-courses are described most easily by plotting them against the logarithm of time after the start of the release (Fig. 3 *b*). When plotted in this way, the time-course of the former is approximately linear, but for the latter it is concave up. The recovery has been characterized by measuring two parameters (Fig. 3 *a*): first, the initial rate of recovery from the slope of the oscilloscope trace 15 ms after the start of the release, and second, the active half response time, defined here as the time taken for the wall tension to recover halfway to the wall tension 10 s after the release.

The internal circumference response to an isotonic release (Fig. 3 *c*) had three stages (Johansson et al., 1978; Mulvany, 1978). First, a decrease in internal circumference simultaneous with the drop in wall tension. Second, a further period of rapid shortening, which slowed over a period of about 150 ms to a steady shortening velocity, which was the third stage. These three stages were always seen regardless of the size of release (Fig. 3 *d*). The response was characterized (Fig. 3 *c*) by measuring the initial rate of shortening (15 ms after the start of the release) and the steady rate of shortening (300 ms after the release) from the slopes of the oscilloscope traces.

Effect of Temperature on the Isometric Release Response

The rate of wall-tension recovery after an isometric release was found to be temperature dependent. This was quantified by performing five experiments similar to that described in Fig. 4, where isometric releases of different extents were made first at 37°, then at 27° C, and then again at 37° C. The isometric active wall tension, ΔT_1, developed was essentially independent of temperature (Table I). The initial rate of rise of wall tension, $\dot{T}_i$, was dependent on the extent of release, δL, but was a maximum for releases of 0.021 L_1. This maximum value of $\dot{T}_i$ (4.53 ΔT_1/s at 37° C) had a Q_{10} of 1.47 (Fig. 4 *a*). For a given temperature the active half-response time, $\tau_{1/2}$, was approximately linearly related to the extent of release (Fig. 4 *b*). In each experiment, the data for each temperature was fitted to the equation:

$$\tau_{1/2} = (\tau_{1/2})_0 + \theta \cdot \delta L/L_1. \quad (1)$$

$(\tau_{1/2})_0$ is the extrapolated active half response time for very small releases and was 0.275 s at 37°C and had a Q_{10} of 1.95. The Q_{10} of the regression line slope, θ, was 1.97. Full details are given in Table I.

TABLE I
PARAMETERS OBTAINED FROM FIVE ISOMETRIC RELEASE EXPERIMENTS

Parameter		37°C	27°C	Q_{10}	P
ΔT_1	mN/mm	2.97 ± 0.17	2.84 ± 0.18	—	NS
$(\dot{T}_i)_{max}$	ΔT_1/s	4.53 ± 0.29	3.10 ± 0.25	1.47 ± 0.02	<0.01
$(\delta L)_{max}$	L_1	0.021 ± 0.004	0.021 ± 0.006	—	NS
$(\tau_{1/2})_0$	s	0.275 ± 0.013	0.529 ± 0.037	1/(1.95 ± 0.20)	<0.001
θ	s/($\delta L/L_1$)	9.31 ± 1.17	17.28 ± 0.83	1/(1.97 ± 0.25)	<0.001

$(\dot{T}_i)_{max}$ is the maximum measured initial rate of rise of wall tension, and $(\delta L)_{max}$ is the extent of release that produced this. $(\tau_{1/2})_0$ and θ are, respectively, the ordinate intercept and slope of the regression of active half response time, $\tau_{1/2}$, on extent of release, δL, as given by Eq. 1 in the text. *P* shows significance of difference between measurements made at 37°C and at 27°C. Average value of L_1/π was 172 μm. Mean active media stress, $\Delta\sigma_1$, was 204 mN/mm^2.

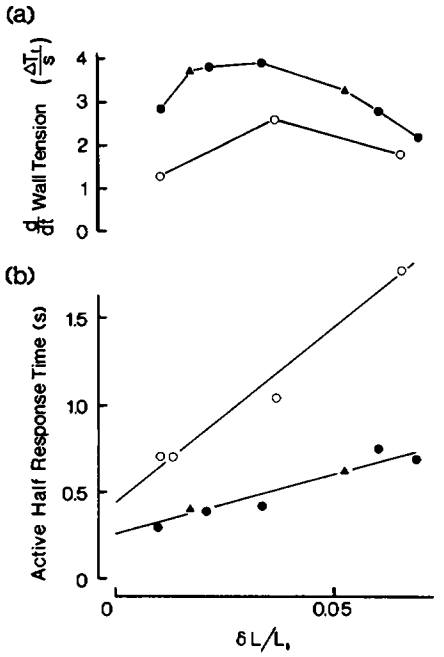

FIGURE 4

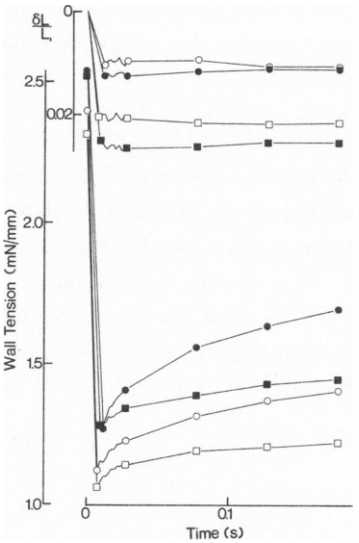

FIGURE 5

FIGURE 4 Experiment showing effect of temperature and extent of release of isometric release response of an activated vessel. Each point refers to one release. Vessels were subjected to a series of releases at 20-min intervals (being relaxed between activations, see text) first at 37°C (filled circles), than at 27°C (open circles) and then again at 37°C (triangles). Upper panel (*a*) shows initial rate of wall tension recovery, $\dot{T}_i$, (see Fig. 3*a*) plotted against extent of internal circumference releases, δL. Lower panel (*b*) shows active half response time, $\tau_{1/2}$, plotted against δL. The lines are regression lines fitted to the points at each temperature. L_1/π was 171 μm; ΔT_1 was 3.23 mN/mm.

FIGURE 5 Comparison of initial part of isometric release responses activated at L_1 (circles) and when relaxed in Ca-free solution and stretched to give a resting wall tension about equal to the active wall tension at L_1 (squares). The releases were performed at 37°C (filled symbols) and 27°C (open symbols). The responses were here measured digitally at 2-ms intervals for the first 28 ms of the release, and thereafter at 50 ms intervals. The lines join measured points. The upper curves show measurements of the imposed change in circumference, the lower curves the measurements of the tension responses. The extent of release was adjusted in each case so that the wall-tension drop was about 0.5 ΔT_1. Note that (*i*) a greater release was required in the stretched vessel than in the activated vessel, (*ii*) the rate of wall-tension recovery was greater in the activated vessel than in the stretched vessel, and (*iii*) the rate of recovery was temperature dependent in the activated vessel but not in the stretched vessel. ΔT_1 = 2.47 mN/mm; L_1/π = 184 μm.

For comparison, the initial rate of rise of wall tension was measured at 37°C and 27°C in relaxed vessels (held in Ca-free PSS to eliminate any possibility of activity) when stretched to give a steady resting wall tension equal to ΔT_1 (Fig. 5). Here the initial rate of rise of wall tension was less than that found in the active vessel and not so temperature dependent. It will be noted that the dynamic stiffness (the ratio of the intial drop in wall tension to δL) is less in the stretched vessel than in the activated vessel, and also that the extent of recovery is less in the stretched vessel. However, the very existence of a substantial recovery in the stretched and relaxed vessel emphasizes the importance of investigating the properties of active vessels at an

internal circumference for which the resting wall tension is negligible in comparison to the active wall tension. It was for this reason that in the experiments reported in this investigation, the releases were made from internal circumference L_1 where the resting wall tension was $<5\%$ of the active wall tension, even though the maximum active wall tension was obtained at about 1.1 L_1, with this active tension being about 5% greater than ΔT_1 (Mulvany and Halpern, 1977).

Effect of Temperature on the Isotonic Release Response

The temperature dependence of the isotonic response was investigated in four experiments similar to that shown in Fig. 6. In these experiments, the effect of the extent of release on the initial rate of shortening, V_i, and the steady shortening velocity, V_s, were determined at 37°C and 27°C. The ratio V_i/V_s was found to be independent of the extent of release (Fig. 6). The mean values obtained in each experiment are shown in Table II: the mean value at 37°C (6.4) was on average slightly lower than the mean value at 27°C (7.3). The relation between V_s and the isotonic load, T_r, was fitted to Hill's equation,

$$(T_r + a) \cdot (V_s + b) = \text{constant}, \tag{2}$$

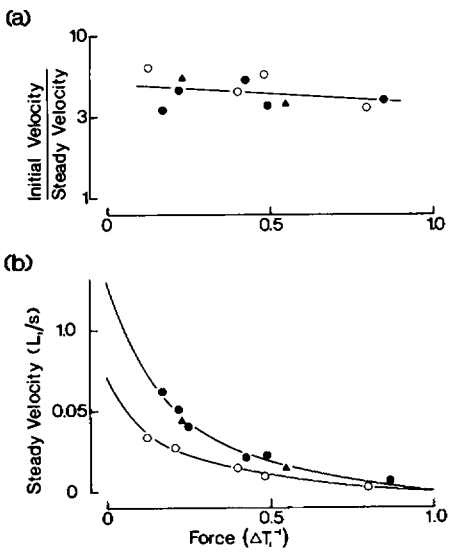

FIGURE 6

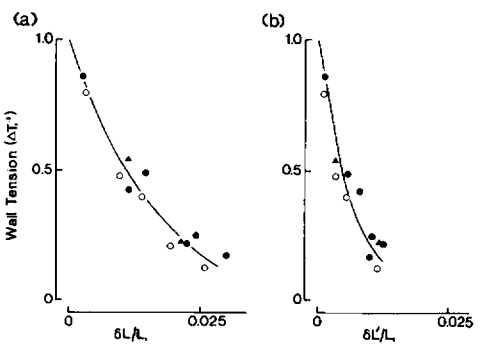

FIGURE 7

FIGURE 6 Experiment showing effect of temperature and extent of release on isotonic release responses of an activated vessel. Each point refers to one release. Vessels were subjected to a series of releases in the same way as in the experiment described in Fig. 4, first at 37°C (filled circles), then at 27°C (open circles) and then again at 37°C (triangles). Upper panel (*a*) shows ratio of initial, V_i, to steady, V_s, shortening velocities plotted against isotonic load, T_r (see Fig. 3*c*). Lower panel (*b*) shows V_s plotted against T_r. The points at each temperature have been fitted to Hill's equation (see text) with the curves shown. $\Delta T_1 = 2.36$ mN/mm; $L_1/\pi = 186$ μm.

FIGURE 7 Series elastic characteristics determined in the isotonic releases described in Fig. 6. Each point refers to one release and shows the isotonic load, T_r, plotted against (*a*) δL, (*b*) $\delta L'$, where δL, $\delta L'$ are as defined in Fig. 3*d*. Symbols are as in Fig. 6. The points have been fitted to Eq. 3 in the text with the curves shown.

TABLE II
PARAMETERS OBTAINED FROM FOUR ISOTONIC RELEASE EXPERIMENTS

Parameter		37°C	27°C	Q_{10}	P
ΔT_1	mN/mm	3.30 ± 0.60	2.90 ± 0.50	—	NS
a	ΔT_1	0.23 ± 0.01	0.25 ± 0.05	—	NS
b	L_1/s	0.031 ± 0.002	0.016 ± 0.003	1.94 ± 0.17	<0.01
V_{max}	L_1/s	0.133 ± 0.006	0.068 ± 0.003	1.97 ± 0.18	<0.001
V_i/V_s		6.4 ± 0.5	7.3 ± 0.4	—	NS
p_U	L_1	0.016 ± 0.001	0.013 ± 0.002	—	NS
q_U	L_1	0.005 ± 0.002	0.006 ± 0.003	—	NS
p_D	L_1	0.011 ± 0.002	0.008 ± 0.002	—	NS
q_D	L_1	−0.003 ± 0.002	−0.001 ± 0.002	—	NS

a and b are the constants of Hill's equation (Eq. 2 in the text) fitted to the isotonic load–steady shortening velocity data. V_{max} is the extrapolated value of the maximum steady shortening velocity ($= b/a$). V_i/V_s is the ratio of the initial to the steady shortening velocity. p_U, q_U, p_D, and q_D are the constants of Eq. 3 in the text describing the form of the undamped (subscript U) and the damped (subscript D) elastic characteristics.

for each experiment. Constant a was essentially independent of temperature but constant b had a Q_{10} of 1.94. Thus for any given isotonic load, both V_s and V_i were about two times greater at 37°C than at 27°C. The extrapolated values of $(V_i)_{max}$ and $(V_s)_{max}$ at 37°C (the values of V_i and V_s for $T_r = 0$) were 0.83 L_1/s and 0.13 L_1/s.

The "Undamped" and "Damped" Series Elastic Characteristics

For each isotonic release the initial change in internal circumference, δL, and a constructed parameter, $\delta L'$, have been determined (Fig. 3 *a*). The quantity $\delta L'$ is obtained by back-extrapolating the steady shortening velocity to time zero and taking $\delta L'$ as the difference between this point and the internal circumference at the end of the first stage. The dependencies of δL and $\delta L'$ on the isotonic release load are, for reasons which will become apparent, here described as the undamped and damped series elastic characteristics, respectively. Both characteristics were essentially independent of temperature and were nonlinear (Fig. 7). They could be fitted (Table II) by equations of the form

$$\delta L \text{ or } \delta L' = p \cdot \ln\left(\frac{T_r}{\Delta T_1}\right) + q \cdot \left(1 - \frac{T_r}{\Delta T_1}\right), \tag{3}$$

where p and q are constants. In the four experiments the slopes of the curves fitted to the points obtained at 37°C were, for $T_r = \Delta T_1$, 49 $\Delta T_1/L_1$ and 127 $\Delta T_1/L_1$, respectively.

DISCUSSION

In skeletal muscle the response to both isometric and isotonic releases have four well-defined stages (Huxley, 1974). In the isometric release response, there is first a decrease in tension simultaneous with the release, second, a period of rapid recovery with a short time-constant (~2 ms in frog fast fibers at 0°C), third, a period of steady tension (or tension fall), and fourth, a period of full recovery. In the isotonic release response there is first a decrease in length simultaneous with the release, second, a period during which the rate of shortening decreases, third, a period of little length change (or even lengthening), and fourth, a period of steady shortening (obeying Hill's equation).

In smooth muscle the time resolution of isometric and isotonic release experiments has not until recently been sufficient to describe adequately the form of the transient changes involved. It is, however, generally agreed (Murphy, 1976) that, in comparison with skeletal muscle fibers, the characteristics of the undamped-SEC is less steep (and nonlinear) and also that in isotonic releases, although the force-steady velocity relation can be fitted by Hill's equation, the velocity of shortening is much slower. The experiments described in this paper have been done with a greater time resolution (4 ms), but have in general confirmed these reports. The experiments have, however, been mainly concerned with investigating the transient responses after isometric and isotonic releases. The isotonic release response has been found to have a velocity transient (Johansson et al., 1978; Mulvany, 1978), somewhat similar to that found in skeletal muscle fibers, but it is slower and more extended and does not contain the third stage of little length change. The isometric release response also differs from that of skeletal muscle fibers in that the recovery is slower and monotonic and does not apparently contain either the second or third stages found in skeletal muscle fibers. It is of course possible that these "missing" stages would be revealed if the vessels were subjected to still faster releases. However, the form of the responses observed (Fig. 3) suggests that this is not the case.

In the skeletal muscle fiber experiments described above all four stages of the isometric and isotonic release responses have been ascribed to the transient characteristics of the force-generating apparatus within them, the cross-bridges between the contractile filaments. In particular, the second stage has been attributed to conformational changes occurring in the attached cross-bridges (Huxley and Simmons, 1971) or to transient changes in their rate of attachment and detachment (Civan and Podolsky, 1966; Abbott and Steiger, 1977). Such interpretations have only been justified because the contractile apparatus is organized in a regular sarcomere structure, so that it has been reasonable to assume that changes in fiber length are directly imposed on the contractile apparatus. Such assumptions cannot at present be justified in smooth muscle, for the structure is less regular. Although it is now generally thought that the force produced by smooth muscle is the result, as in skeletal muscle, of the interaction of myosin cross-bridges and actin, both the actin and myosin being arranged in filaments (Lowy et al., 1970; Somlyo et al., 1973), the contractile filaments do not appear to be arranged in uniform sarcomeres (Ashton et al., 1975; Small, 1977). The means by which the force is transmitted from the contractile filaments to the cell membrane is not known, nor is the mechanism of force transmission between cells understood, although this may be the result of the cells being enmeshed in a collagen matrix (Gabella, 1977). It is therefore unlikely that the transient isometric and isotonic responses reported in this paper are a direct expression of the mechanisms of the smooth muscle cross-bridges. On the other hand, it is clear that both responses, taken as a whole, are the result of active processes: after isometric releases there is full tension recovery, while isotonic releases result finally in a steady velocity of shortening. Thus it seems probable that the form of the transients is also, at least in part, dependent on the properties of the contractile apparatus. This interpretation is supported by the finding (Tables I, II) that the form of the early parts of the isometric and isotonic responses of active vessels (but not of relaxed vessels—Fig. 5) is temperature dependent with Q_{10}'s similar to that of the steady shortening velocity.

The primary purpose of the experiments described in this paper has been to show how the

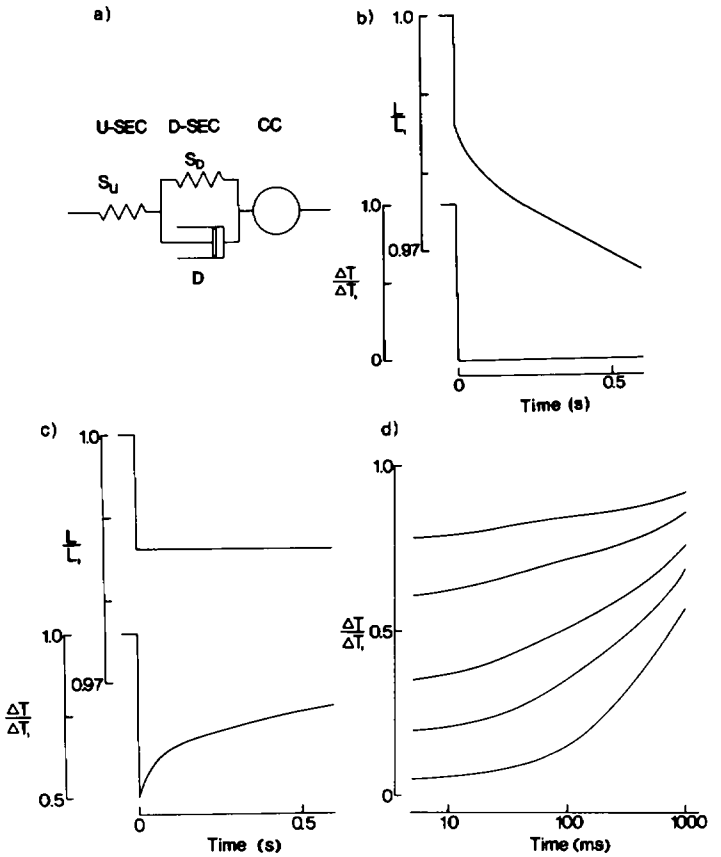

FIGURE 8 Characteristics of model describing mechanical behavior of activated vascular smooth muscle. (a) Configuration of model components, being the series combination of an undamped-SEC (U-SEC), a damped-SEC (D-SEC) and a (CC). The components have the following equations of motion:

$$S_U : \dot{x} = (p_U/\Delta T + q_U) \cdot \Delta \dot{T}. \quad \text{(i)}$$

$$S_D : \dot{y} = (p_D/T_S + q_D) \cdot \dot{T}_s. \quad \text{(ii)}$$

$$D : \dot{y} = \mu \cdot T_D. \quad \text{(iii)}$$

$$\text{CC} : (\Delta T + a)(\dot{z} + b) = \text{constant}. \quad \text{(iv)}$$

Eqs. i and ii are the differential forms of Eq. 3 in the text. Eq. iii is the characteristic of a dashpot with coefficient of damping μ. Eq. iv is Hill's equation, Eq. 2 in the text. x, y, and z are the lengths of the U-SEC, D-SEC, and CC, respectively and $x + y + z = L$, the overall length. T_S and T_D are, respectively, the instantaneous tensions in spring S_D and dashpot D. $T_S + T_D = \Delta T$, the total tension developed by the CC. The initial values of L and ΔT are L_1 and ΔT_1, respectively. The model parameters, p_U, p_D, q_U, q_D, a, and b are set to the experimentally determined values obtained in isotonic releases and reported in Table II, i.e., $p_U = 0.0161\ L_1$; $q_U = 0.0048\ L_1$; $p_D = 0.0107\ L_1$; $q_D = -0.0025\ L_1$; $a = 0.23\ \Delta T_1$, and $b = 0.031\ L_1$/s. The parameter μ is set 4.36 $L_1/(\text{s} \cdot \Delta T_1)$ to give $V_l/V_s = 6.4$ for a release to $\Delta T = 0.5\ \Delta T_1$ (Table II). (*b*) Form of model isotonic release response. Note the presence of a velocity transient after release (compare with Fig. 3 *c*). (*c*) Form of model isometric release response. Note that tension recovery increases monotonically with time (compare Fig. 3 *a*). (*d*) Model isometric release responses for releases of (from top) 0.005 L_1, 0.01 L_1, 0.02 L_1, 0.03 L_1, and 0.04 L_1 plotted with time on a logarithmic scale. Note that for small releases the curves are approximately linear, but that for large releases they are concave up (compare with Fig. 3*b*).

SEC of smooth muscle can, as in skeletal muscle, be considered as the series connection of an undamped- and a damped-SEC (Fig. 1 *b*). It is clear from the form of the isotonic release response (Fig. 3) that it could be described by such a model but, as is shown in Fig. 8, by using the parameters determined from the isotonic release response, the model also describes both qualitatively and quantitatively the isometric release response. That is, an isometric release of the model results in a monotonic tension recovery that for small releases is approximately linear with respect to the logarithm of time after the start of the release, but that is concave up for larger releases. Thus the modified Hill model shown in Fig. 1 *b*, which provides approximate description of the responses of skeletal muscle to isotonic and isometric releases, will also with suitable parameters provide reasonable descriptions of the responses to such releases in smooth muscle. It should be noted that the inclusion of a contractile component in the model makes it unnecessary for the time-constant of the damped-SEC to be tension dependent, as proposed by Greven (1976).

Finally, it must be emphasized that the division of the SEC into undamped and damped parts is a mathematical description, in the same way that Hill's original description was, and does not imply a morphological location for these components. The extent to which these form part of the contractile apparatus, the passive structures between this and the cell membrane or the intercellular connections, remains to be determined.

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REFERENCES

ABBOTT, R. H., and G. J. STEIGER. 1977. Temperature and amplitude dependence of tension transients in glycerinated skeletal and insect fibrillar muscle. *J. Physiol. (Lond.).* **266**:13–42.

ALEXANDER, R. S. 1976. Series elasticity of urinary bladder smooth muscle. *Am. J. Physiol.* **231**:1337–1343.

ASHTON, F. T., A. V. SOMLYO, and A. P. SOMLYO. 1975. The contractile apparatus of vascular smooth muscle: Intermediate high voltage stereo electron microscopy. *J. Mol. Biol.* **98**:17–29.

BRESSLER, B. M., and N. F. CLINCH. 1974. The compliance of contracting skeletal muscle. *J. Physiol. (Lond.).* **237**:477–494.

BURR-BROWN. 1966. Handbook of Operational Amplifiers. 68–69.

CIVAN, M. M., and R. J. PODOLSKY. 1966. Contraction kinetics of striated muscle fibres following quick changes in load. *J. Physiol. (Lond.).* **184**:512–534.

COX, R. H. 1977. Determination of the series elasticity in arterial smooth muscle. *Am. J. Physiol.* **233**:*H*248–*H*255.

DOBRIN, P., and T. CANFIELD. 1977. Identification of smooth muscle series elastic component in intact carotid artery. *Am. J. Physiol.* **232**:*H*122-*H*131.

FAY, F. S. 1977. Isometric contractile properties of single isolated smooth muscle cells. *Nature (Lond.).* **265**:553–560.

FORD, L. E., A. F. HUXLEY, and R. M. SIMMONS. 1977. Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *J. Physiol. (Lond.).* **269**:441–515.

GABELLA, G. 1977. Arrangement of smooth muscle cells and intramuscular septa in the taenia coli. *Cell Tissue Res.* **184**:195–212.

GREVEN, K. 1976. The time course of creep and stress relaxation in relaxed and contracted smooth muscles (taenia coli of the guinea pig). Physiology of Smooth Muscle. E. Bülbring and M. F. Shuba, editors. Raven Press, New York. 223–228.

HERLIHY, J. T., and R. A. MURPHY. 1974. Force-velocity and series elastic characteristics of smooth muscle from hog carotid artery. *Circ. Res.* **34**:461–466.

HILL, A. V. 1938. The heat of shortening and the dynamic constants of muscle. *Proc. Roy. Soc. Lond. B. Biol. Sci.* **126**:136–195.

HILL, A. V. 1970. First and Last Experiments in Muscle Mechanics. Cambridge University Press, Cambridge, England. 85–109.

HUXLEY, A. F. 1974. Review lecture, muscular contraction. *J. Physiol. (Lond.).* **243:**1–44.

HUXLEY, A. F., and R. M. SIMMONS. 1971. Proposed mechanism of force generation in striated muscle. *Nature (Lond.).* **233:**553–538.

JEWELL, B. R., and D. R. WILKIE. 1958. An analysis of the mechanical components in frog's striated muscle. *J. Physiol. (Lond.).* **143:**515–540.

JOHANSSON, B., P. HELLSTRAND, and B. UVELIUS. 1978. Responses of smooth muscle to quick load change at high time resolution. *Blood Vessels.* **15:**65–82.

LOWY, J., F. R. POULSEN, and P. J. VIBERT. 1970. Myosin filaments in vertebrate smooth muscle. *Nature (Lond.).* **225:**1053–1054.

MEISS, R. A. 1978. Dynamic stiffness of rabbit mesotubarium smooth muscle: effect of isometric length. *Am. J. Physiol.* **234:***C*14–*C*26.

MULVANY, M. J. 1978. Response of vascular smooth muscle to rapid load changes. *J. Physiol. (Lond.).* **280:**28–29P.

MULVANY, M. J., and W. HALPERN. 1976. Mechanical properties of vascular smooth muscle cells in situ. *Nature (Lond.).* **260:**617–619.

MULVANY, M. J., and W. HALPERN. 1977. Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.* **41:**19–26.

MULVANY, M. J., HANSEN, P. K., and C. AALKJAER. 1978. Direct evidence that the greater contractility of resistance vessels in spontaneously hypertensive rats is associated with a narrowed lumen, a thickened media and an increased number of smooth muscle cell layers. *Circ. Res.* **43:**854–864.

MURPHY, R. A. 1976. Contractile system function in mammalian smooth muscle. *Blood Vessels* **13:**1–23.

SMALL, J. V. 1977. Studies on isolated smooth muscle cells: the contractile apparatus. *J. Cell Sci.* **24:**327–350.

SOMLYO, A. P., C. E. DEVINE, A. V. SOMLYO, and R. V. RICE. 1973. Filament organization in vertebrate smooth muscle. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* **265:**223–229.

STEPHENS, N. L., and U. KROMER. 1971. Series elastic component of tracheal smooth muscle. *Am. J. Physio.* **220:**1890–1895.