The Active Tension-Length Curve of Vascular Smooth Muscle Related to Its Cellular Components

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ABSTRACT The active and passive isometric tension-length (internal circumference) relation of vascular smooth muscle has been investigated using a 100–200-μm lumen diameter artery from the rat mesenteric bed. Conditions were established under which maximal activation was obtained at all lengths. Below \( L_0 \) (the length at which maximum tension, \( \Delta T_0 \), was developed) the active tension fell with decreasing length along a line which extrapolated to 0.38 \( L_0 \); below 1.1 \( L_0 \) the relation was reversible regardless of the protocol used. Above \( L_0 \) the active tension fell linearly with increasing length along a line which extrapolated to zero tension at 1.82 \( L_0 \). At the longer lengths investigated (up to 1.6\( L_0 \)) the passive tension upon which the active responses were superimposed was as high as 4.4 \( \Delta T_0 \). However, measurements of the dynamic characteristics of the preparation (with a time resolution of 2 ms) suggest that the active tension measured is nevertheless a measure of the active properties of the contractile apparatus. Direct light microscopic observation of the effect of length change on the cells within the walls of the preparation showed that changes in vessel length produced, on average, the same percentage change in cell length. Histological examination showed no signs of cell destruction following large extensions. The results suggest that the decrease in tension with extension above \( L_0 \) is due to changes in the properties of the contractile apparatus, rather than to cellular damage.

INTRODUCTION

There is now considerable structural evidence that the contractile proteins in vertebrate smooth muscle, as in skeletal muscle, are arranged in filaments (Lowy et al., 1973; Ashton et al., 1975). Thus by analogy with skeletal muscle (Gordon et al., 1966), it might be expected that the amount of overlap between the contractile filaments, and also the active tension, will vary with muscle length. The active tension-length curve of smooth muscle is qualitatively similar to that of skeletal muscle (Herlihy and Murphy, 1973), and therefore, it is tempting to infer that smooth muscle also operates via a sliding filament mechanism. There are, however, a number of reasons for doubting the validity of this inference. First, in skeletal muscle the correlation between active tension development and filament overlap can only be demonstrated precisely in single fibres in which the sarcomere lengths are clamped (Julian et al., 1978). The relatively loose
arrangement of the filaments within smooth muscle cells precludes such precautions in smooth muscle. Second, the cells of smooth muscle are small and held in a mesh, so that is is not even possible to hold the cell length constant during activation. Third, most published work on the active tension-length relation refers almost entirely to the variation of active tension at lengths below $L_0$, the length at which the active tension is a maximum. This is because it is generally found that activation at lengths above $L_0$ results in "damage" to the preparation, so that when subsequently activated at $L_0$ the active tension is irreversibly decreased (Peterson and Paul, 1974). Further, the resting tension of most smooth muscles rises sharply at lengths above $L_0$, making it difficult to distinguish precisely between the active and passive responses. It is, however, the form of the curve above $L_0$ which is of the greatest interest for, in skeletal muscle at least, below $L_0$ impaired excitation-contraction coupling appears to prevent the active tension-length curve providing a true indication of filament overlap (Rüdel and Taylor, 1971).

We have therefore decided to investigate the active tension-length curve of smooth muscle using a small vascular preparation, namely a $150-200\,\mu m$ lumen diameter artery in the mesenteric bed of the rat (Mulvany and Halpern, 1976). This preparation has the advantage that, unlike larger preparations, the smooth muscle cells within the walls can be directly visualized, allowing us to relate changes in muscle length to changes in cell length. Furthermore, under our handling, this preparation shows less irreversible loss of active tension following extension than other investigators have reported for larger preparations. We have therefore been able to investigate a considerable portion of the active tension-length curve above $L_0$. In addition, we have determined the dynamic mechanical properties of the preparation (with a time resolution of $\sim 2\, ms$), and this has enabled us to differentiate more certainly between the active and passive characteristics.

**METHODS**

**Dissection and Mounting**

The preparation used was a 0.7 mm segment of a small (100-200-\mu m lumen diameter) arterial resistance vessel taken from the mesenteric bed of 3-5-mo-old Wistar rats. The procedures for dissection and mounting of the preparation have been described previously (Mulvany and Halpern, 1977). Recent improvements to the myograph (Fig. 1) consisted of reducing the size of the preparation supports, linearizing the motion of the piezoelectric pusher (PZ40, Burleigh Instruments Inc., Fishers, N.Y.) and measuring this motion with a new high resolution (50-nm) displacement transducer (KD-2810-IU, Kaman Sciences Corp., Colorado Springs, Colo.). The myograph had a frequency response of 600 Hz with an overall compliance of about 0.5 \mu m/mN. This compliance corresponds to $\sim 0.005\, L_0\, \Delta T_0$ (see below) and has not been corrected for. In the experiments described in this paper, the myograph has been used to make direct measurements of wall tension, $T$, while the internal circumference, $L$, was controlled. Measurements of $T$ and $L$ were recorded on-line by a mini-computer (Prime Computer Inc., Framingham, Mass.) with a sampling rate of up to 1 ms$^{-1}$ and on an oscilloscope (D11, Tektronix, Inc., Beaverton, Ore.) and an oscillograph (7402A, Hewlett-Packard...
MULVANY AND WARSHAW Length-Tension Curve of Vascular Smooth Muscle

The computer was also used to initiate isometric releases and to perform analysis of the resulting responses as described in the Appendix.

Normalization and Nomenclature

In this preparation the smooth muscle cells within the walls are oriented circumferentially (Mulvany and Halpern, 1976), so that with the method of mounting used, the “length” of the preparation is the internal circumference. We will therefore describe the tension-length relationship of the preparation as the tension-internal circumference relation.

FIGURE 1. Myograph used in this investigation. Vessel segments were threaded onto 40-μm stainless steel wires which were attached to supports L and R mounted, respectively, on a force transducer (DSC6, Kistler-Morse Corp., Bellevue, Wash.) and a pusher (PZ40, Burleigh). The pusher (range 15 μm) was mounted on a micrometer for gross movements, which was monitored by a 1-μm resolution displacement transducer (KD 2900.5 SU, Kaman Sciences Corp., not shown). The movement of the pusher relative to the micrometer was set according to an output signal from the computer (Prime 300, Prime Computer Inc.) to within 0.05 μm by a feedback system incorporating the high resolution displacement transducer (KD-2810-1U, Kaman Sciences Corp.) as shown. This computer was used to initiate controlled rapid (5-ms) internal circumference changes (Fig. 2) and to record digitally the outputs of the force and displacement transducers for later analysis.

After mounting, vessels were set to an internal circumference $L_1 = 0.8 L_{100}$, where $L_{100}$ was an estimate, made from the passive tension-internal circumference relation of the internal circumference that the vessel would have had in situ when relaxed, and under an internal pressure of 100 mmHg (Mulvany and Halpern, 1977). Subsequent changes in internal circumference were made with reference to $L_1$. At the end of the experiment the internal circumference for which the active tension development had been a maximum was determined and denoted $L_0$. $L_0$ was approximately 1.2$L_1$. All internal circumferences are reported in the text with reference to $L_0$.

Passive and active tensions are reported, respectively, as the force measured by the
force transducer in relaxing solution (see below) and the increase in force upon
activation. \( \Delta T_a \) is the active tension measured during the first activation at \( L_0 \).

**Solutions**

The following solutions were used. Physiological salt solution (PSS) contained (in millimolar): \( \text{NaCl}, 119; \text{NaHCO}_3, 25; \text{KCl}, 4.7; \text{KH}_2\text{PO}_4, 1.18; \text{MgSO}_4, 1.17; \text{CaCl}_2, 1.6; \)
ethylenediaminetetraacetic acid (EDTA), 0.026; glucose, 5.5. Activating solution was as
for PSS, but with 40 \( \mu \text{M} \) norepinephrine and with an equimolar exchange of \( \text{NaCl} \) for
\( \text{KCl} \) and with 2.5 \( \text{mM} \) \( \text{CaCl}_2 \). Relaxing solution was as for PSS but without \( \text{CaCl}_2 \) and with
1 \( \text{mM} \) ethylene glycol-bis (\( \beta \)-aminoethyl ether)-\( N,N' \)-tetraacetic acid (EGTA).

The suitability of the activating solution used was assessed by a series of experiments
in which the dose-response characteristics of the vessels to the two agonists in the
activating solution, norepinephrine and potassium, were obtained over a range of
internal circumferences between 0.6 \( L_0 \) and 1.4 \( L_0 \). On the basis of these experiments, we
concluded that the composition of the activating solution was such that maximal
activation could be expected at all internal circumferences and that any variations in
response could be ascribed to length dependent variations in the behavior of the vessels'
contractile apparatus rather than the degree of activation. The effect of repeated
activations with activating solution was then investigated. Vessels were activated regularly
at 15-min intervals for 3 min with activating solution with the vessels held continously at
0.8 \( L_0 \). In all cases we found that the responses remained constant (within \( \pm 5\% \)) in
experiments involving up to 30 activations (and thus extending over 8 h), apart from the
initial response which was always lower. After normalization, experiments therefore
always included an initial activation at 0.8 \( L_0 \), the response to which was not included in
the analysis.

All solutions were bubbled with 95%/5% \( \text{O}_2/\text{CO}_2 \) and adjusted to pH 7.4. Experiments
were performed at 37°C.

**In Vitro Microscopy**

The myograph was mounted on a microscope (Carl Zeiss, Inc., New York; Photomicro-
scope II) equipped with Nomarski interference contrast optics (Mulvany and Halpern,
1976). Under these conditions the smooth muscle cells in the media could be visualized in
longitudinal section (between the mounting wires). This ability has been used to estimate
changes in cell length under various conditions by measuring the change in the spacing
of intracellular features (Halpern et al., 1978). The measurements were made from
photographic records (made on Kodak high contrast film, Eastman Kodak Co., Roches-
ter, N.Y.), by projecting the negatives onto a matt screen to give a final linear
magnification of 4,250.

**Histology**

Vessels were examined histologically after fixation either while activated (held in
activating solution) or while relaxed (held in relaxing solution). Vessels were fixed by
draining the test solution and filling the chamber with prefix (2.5% glutaraldehyde in
cacodylate buffer, pH 7.4). After at least 30 min vessels were removed from the
myograph and held in prefix (at 4°C) until the remainder of the fixation procedure was
carried out. This procedure (Mulvany et al., 1978) involved postfixing in \( \text{OsO}_4 \),
blockstaining in uranylacetate, dehydration in ethanol, and embedding in Epon (Shell
Chemical Co., New York) Thick sections (1 \( \mu \text{m} \)) for light microscopy were stained with
toluidene blue and photographed with Pan-F film (Eastman Kodak Co.).
**Experimental Procedure**

Following normalization and the initial conditioning activation, vessels were subjected at 12-min intervals to the procedure shown in Fig. 2. The bathing solution was changed in turn for 2 min to (a) relaxing solution, (b) activating solution, and (c) relaxing solution, after which the solution was changed back to PSS for the remaining 6 min of the cycle. The passive tension was taken as the mean of the recorded tensions at the end of the two periods in relaxing solution. The active tension was taken as the difference between the tension recorded at the end of the period in activating solution and the passive tension. This procedure was adopted to allow for the slight stress relaxation which is present in the relaxed vessels, particularly at the longer lengths. The internal circumference was changed to a new value (if required) at the start of the period in PSS.

At the end of each of the periods in the test solutions (a), (b), and (c), vessels were
subjected to 1% isometric releases to determine their dynamic properties. From these, as
described in the Appendix, we have determined the passive isometric release response
and the active isometric release response. These have been characterized by determining
for each response the initial rate of recovery and the extent of recovery after 10 s
(Mulvany, 1979). We have also used the responses to determine the passive and active
dynamic stiffnesses as described in the Appendix.

RESULTS

Morphological and Histological Observations

We have utilized the ability to see cells within the walls of our arterial resistance
vessel preparation to observe directly the effects on cells of both extension and
activation. In five experiments similar to that shown in Fig. 3 a, we have
followed (and recorded photographically) the change in spacing of intracellular
features over the range \( L_0 \) to 1.6 \( L_0 \) for vessels in PSS. The results are presented
in Fig. 3 b and suggest that, over this range, changes in vessel internal
circumference were accompanied, on average, by almost the same percent
changes in cell length. The considerable dispersion of the measurements
suggests, however, that there may be significant variation in the effect of stretch
on individual cells. Measurements were not made below \( L_0 \) due to lack of cell
definition at such internal circumferences.

Observations of cells during activation revealed substantial relative movement.
Intracellular features could be seen to move by up to 50 \( \mu \)m across the field of
view, i.e., almost one cell length. This movement prevented us making mea-
surements of changes in cell length during activation. However, in some cases
nuclei could be observed and followed during activation, and their length was
found on average to decrease by 7 ± 11% SD (31 measurements on seven nuclei
from three vessels). Thus, although we found no evidence that the average
length of cells was affected substantially by activation, the observations suggest
that there is significant rearrangement of the cells during activation, implying
that some lengthen while others shorten.

The histological effects of stretch on the preparation have been assessed by
fixing vessels either when relaxed or when activated at various internal circum-
fences, and representative examples of the vessels which were fixed while
activated are shown in Fig. 4. Activation below \( L_0 \) was found to produce a
marked distortion of the internal elastic lamina. Above \( L_0 \) the cells are aligned
circumferentially and there is no sign of internal elastic lamina distortion. The
effect of the mounting wires is clearly seen in the vessel fixed at 0.6 \( L_0 \), whereas
in those fixed at the longer lengths their effect is much reduced or insignificant.
However, in all cases the cells which were located in the portion of the wall
which wrapped around the wires, the staining properties of the cells were
unaffected, indicating lack of cellular damage.

Tension–Internal Circumference Relation

In eight experiments active and passive tensions were measured over a range of
internal circumferences between 0.5 \( L_0 \) and 1.6 \( L_0 \). In these experiments vessels
were stimulated at internal circumferences set sequentially to values \((a)\) from 0.8
Figure 3. (a) Interference contrast (Nomarski) micrographs showing change in distance between intracellular markers (arrowed) when a relaxed vessel is set in turn (from top) to $1.0 L_0$ (1.00), $1.62 L_0$ (1.55), $1.50 L_0$ (1.46), $1.25 L_0$ (1.23), and $1.0 L_0$ (0.97). The numbers in parentheses show the relative distance between the markers in each case. (b) Results of 116 measurements made on 15 cells from five relaxed vessels in a manner similar to that shown in a of the relation between cell length (distance between intracellular markers) and internal circumference. The line is a regression line ($r^2 = 0.8$), and has the equation: relative cell length = 0.93 · (relative internal circumference) + 0.09.
As described in Methods all internal circumference changes were made while the vessel was relaxed, the incremental changes being about ±0.1 \( L_0 \). The average results are shown in Fig. 5, and the average characteristics thus obtained are very similar to those obtained in each of the individual experiments, for example, Fig. 6a. During the initial lengthening sequence, sequence a, the active tension rose to a peak \( \Delta T_0 \) (at \( L_0 \)), and then fell to 0.6 \( \Delta T_0 \) at 1.4 \( L_0 \). During the shortening sequence, sequence b, the active tension lay first along, or a little above, the characteristics obtained in sequence a, but then remained on a plateau at 0.8

\[ \Delta T_0 \text{ between } 1.3 L_0 \text{ and } 0.9 L_0, \text{ before falling to } 0.2 \Delta T_0 \text{ at } 0.5 L_0 \text{ along a line which extrapolated to zero tension at } 0.38 L_0. \]

During the second lengthening sequence, sequence c, the active tension lay close to the values obtained during sequence b, and above 1.4 \( L_0 \) the measurements lay along a linear extension of the curve obtained during sequence a, falling to 0.3 \( \Delta T_0 \) at 1.6 \( L_0 \) along a line which extrapolated to zero tension at 1.82 \( L_0 \). The center of the plateau seen during sequences b and c was always close to \( L_0 \), indicating that, under these conditions, there was little plasticity in the active tension-internal circumference characteristic.
Figure 5. (a) Active tension responses of eight vessels determined at internal circumference starting at $0.8L_0$ and then in the order indicated by the arrows. (●) First ascending sequence; (■) descending sequence (dashed line); (▲) second ascending sequence. Each point is the mean of one response from each vessel (obtained if necessary by interpolation) and the bars show SE. (b) Passive tensions upon which the active tension responses shown in a were superimposed. Symbols as in a. Note that the vertical scale is one quarter of that used in a. $L_0/\pi = 208 \pm 9$ μm; $\Delta T_0 = 3.08 \pm 0.38$ mN (means ± SE).

The measured active tensions were superimposed at lengths above $L_0$, on substantial passive tensions (Fig. 5b). During sequence a the passive tension rose to $2.4 \Delta T_0$ (at $1.4L_0$). This stretch produced irreversible changes in the passive characteristic, for, during the subsequent release sequence b and stretch sequence c, the characteristic was shifted to the right. At $1.6L_0$, the final point
on sequence c, the passive tension was 4.4 $\Delta T_0$, over 15 times the active tension measured at that internal circumference.

**Effect of Different Stretch Sequences**

It has been a common finding of investigators of smooth muscle that the form of the active tension-length characteristic is dependent on the sequence in which the points on the characteristic are obtained. We have therefore conducted a further series of 15 experiments in which different sequences were used. A number of representative examples are shown in Fig. 6 b-f in comparison to a standard protocol experiment, Fig. 6 a. The peak on the active tension-internal circumference curve was retained if vessels were not extended beyond 1.1 $L_0$ (Fig. 6 b). Similarly, if the experiment was started with a shortening sequence, from 0.8 $L_0$ to 0.5 $L_0$ (Fig. 6 c) the peak was present during the subsequent lengthening sequence. Thus, the characteristic only became irreversible follow-
ing extension above 1.1 $L_0$, and it appeared that the amount of active tension which could then be recovered was dependent on the degree of extension (Fig. 6 d). Furthermore, the extent of recovery could not be increased by repeated activations at, for example, $L_0$ (Fig. 6 e). Fig. 6 d shows also that although, as noted above, extension up to 1.4 $L_0$ did not produce significant plasticity in the active tension-internal circumference characteristic, extension above 1.4 $L_0$ did result in a clear rightward shift of the characteristic. Fig. 6 f shows that if, following activation at 1.1 $L_0$, vessels were extended to 1.4 $L_0$ and then shortened to $L_0$ (without activation), the active tension at $L_0$ was not greatly reduced. Thus, it appeared that it was activation of vessels beyond 1.1 $L_0$ which caused the irreversibility, and not just the extension of the vessels.

**Variation of Dynamic Characteristics with Internal Circumference**

As a means for controlling whether our protocol enabled us to make a clear distinction between the active and passive properties of the preparation, we have also measured the dynamic properties of vessels over the range of internal circumferences reported above. This has been done by subjecting vessels at each internal circumference to isometric releases during activation both before and after activation in relaxing solution (Fig. 2). From these we have determined the active and passive isometric release responses (IRRs). As described in the Appendix, the passive IRR is the mean of the IRRs measured in relaxing solution before and after the activation, and the active IRR is the difference between the IRR measured in activating solution and the passive IRR.

The forms of the active and passive IRRs during a typical experiment are shown in Fig. 7. At all degrees of extension it was found that the active IRR had a rapid initial rate of rise, $r$, and showed about 90% recovery, $\eta$, after 10 s. By contrast the passive IRR had a slower initial rate of rise and only showed about 30% recovery after 10 s. The average results from three experiments similar to those described in Fig. 5 are shown in Fig. 8. Above $L_0$ the active values of $r$ and $\eta$ remained essentially constant and two to three times greater than the corresponding passive values. Below $L_0$ the active values of $\eta$ remained constant but that of $r$ increased. The passive tension below $L_0$ was too small for the passive IRR to be measured accurately.

We have also used the IRRs to determine the effect of internal circumference on the ratios of active dynamic stiffness to active tension and of passive dynamic stiffness to passive tension. Above $L_0$ the active dynamic stiffness was proportional to active tension, so that their ratio remained constant. Below $L_0$ the active dynamic stiffness fell more slowly than active tension, so that their ratio increased. The ratio of passive dynamic stiffness to passive tension above $L_0$ was also essentially constant, but less than half the corresponding active ratio. Again, the passive tension below $L_0$ was too small for reliable measurements to be made of the ratio of passive stiffness to passive tension.

**Discussion**

**The Active Tension-Internal Circumference Relationship**

It has long been considered that smooth muscle has a greater working range than skeletal muscle (Winton, 1926). Thus, whereas skeletal muscle fibers,
under conditions of maximal activation, can contract down to 0.5 $L_0$ (Rüdel and Taylor, 1971), vascular smooth muscle strips are able to contract to 0.3-0.4 $L_0$ (Speden, 1960; Herlihy and Murphy, 1973; Peterson and Paul, 1974), where $L_0$ is the length at which maximum tension is developed. Visceral smooth muscle can contract even further: rabbit taenia coli (Gordon and Siegman, 1971), canine bronchus (Stephens et al., 1968), rabbit bladder (Uvelius, 1976), and rabbit mesotubarium (Meiss, 1978) have all been shown to be capable of contracting to about 0.2 $L_0$, as have isolated visceral smooth muscle cells (Bagby and Fisher, 1973; Fay, 1975). The extent of the smooth muscle working range above $L_0$ has not though been studied in detail. This is largely, no doubt, because the passive tension at such lengths is very high and thus tends to mask the active responses.

![Figure 7](https://example.com/figure7.png)

**Figure 7.** Active (A) and passive (P) isometric release responses of vessels to 2-$\mu$m releases (0.008 $L_0$), determined as described in the Appendix for the experiment shown in Fig. 6 a. Such isometric release responses were determined at all internal circumference settings where active and passive tensions were recorded. The responses shown here were obtained in the order indicated a-f. In each case the abscissa shows zero tension. Note that the active responses all have a rapid initial rate of recovery and achieve ~80% recovery in tension after 2 s, while the passive responses have a slower initial rate of recovery and only achieve ~20% recovery after 2 s.

The available evidence (Speden, 1960; Herlihy and Murphy, 1973; Paul and Peterson, 1975) has suggested, however, that the active tension of vascular smooth muscle falls rapidly above $L_0$, so that its range here is less than that of skeletal muscle which develops active tension up to 1.8 $L_0$ (Gordon et al., 1966), although invertebrate smooth muscle appears to have a similar range to skeletal muscle (Cornelius and Lowy, 1978).

The findings of the present investigation are in general agreement with the above results as regards the range of vascular smooth muscle below $L_0$, but suggest that the range above $L_0$ may be greater than previously indicated. The results show that our preparation can develop active tension in the range from 0.38 $L_0$ to 1.82 $L_0$ (Fig. 5 a). Up to 1.1 $L_0$ the characteristic was fully reversible (Fig. 6 b), but was only partially reversible following greater degrees of stretch.
Thus, as other investigators have found (Gordon and Siegman, 1971; Lowy and Mulvany, 1973; Peterson and Paul, 1974), above \( L_0 \) the active tension-length curve is not always reversible, but the degree of reversibility which we have observed seems greater than has previously been reported.

Fig. 8. Variation of dynamic constants of active and passive isometric release responses with internal circumference. (a) Initial rate of recovery, \( r \); (b) recovery after 10 s, \( \eta \); (c) ratio of dynamic stiffness to tension, \( k/T \), were determined in experiments similar to those described in Fig. 5. Points obtained: (O) during first extension sequence; (■) during shortening sequence; (▲) during second extension sequence. Solid symbols refer to parameters obtained from active isometric release responses, empty symbols to those obtained from passive isometric release responses. Each symbol is the mean of values obtained in three experiments, and bars show SE (where this exceeds the size of the symbol). The lines join mean values obtained at each internal circumference. All values were normalized with respect to the initial parameter values obtained at \( L_0 \): 

\[
\begin{align*}
r_0 &= 18 \text{s}^{-1}; \\
\eta_0 &= 0.95; \\
(k/T)_0 &= 44 \text{L}_0^{-1} \text{ (means ± SE).}
\end{align*}
\]

The high degree of passive tension encountered above \( L_0 \) suggests that the lack of recovery could be the result of cellular destruction, which would imply that the measured active responses would be lower than would be expected from intact preparations. However, we found no evidence of any such destruction in sections of vessels fixed after such stretch (Fig. 4). Moreover, the lack of recovery was in contrast to the repeatability of the responses following such
extension (Fig. 5a) and to the constancy of responses observed in repeated stimulations at the same length (see Methods). Therefore, we suggest that extension above $L_0$ does not destroy cells, but that the decrease in active tension at such internal circumferences and the subsequent lack of recovery are due to intracellular changes in the contractile apparatus.

Controls
The active tensions reported above are, as described in Methods, obtained by subtracting the tension measured in relaxing solution from the tension measured in activating solution. The legitimacy of equating the active tension thus determined to the active properties of the contractile apparatus then depends on whether a "Maxwell" or "Voigt" model is assumed for the passive and active elements of smooth muscle (Simmons and Jewell, 1974). In the former case the contractile apparatus is entirely parallel connected with the components which account for the properties of the relaxed preparation. In the latter case, some of these components lie in series with the contractile apparatus. The heterogeneous nature of smooth muscle morphology implies that it is unrealistic to suppose that either model can provide a complete description of the arrangement of the active and passive components. However, the fact that the form and time-course of both the active and passive isometric release responses remained remarkably constant and distinct from each other at all lengths above $L_0$ (Figs. 7 and 8) suggests that a Maxwell model provides an adequate description of the general arrangement of the active and passive structure in our preparation (Dobrin, 1978). Thus, we believe that the active tension measurements that we have made are a reasonable indication of the properties of the active components within the preparation. This conclusion supports a similar conclusion made by Paul and Peterson (1975) who showed that in bovine mesenteric vein the decrease in active tension above $L_0$ was accompanied by a decrease in the suprabasal oxygen consumption.

Another possible source of error in our determination of the active tension-internal circumference characteristic is the method by which our preparation is mounted. Although the method has the advantage that the small size of the preparation enables us to correlate its properties to those of the cells within it, it has the disadvantage that a considerable proportion is in contact with the mounting wires. However, we found no evidence that the wires have in any way damaged the cells; the preparation remains viable for at least 12 h, and the staining properties of vessels fixed at that time show no differences between those cells which had been wrapped around the wires and those which had been situated between them (Fig. 4). We do find, however, that activation results in a thinning of the walls around the wires (Mulvany et al., 1978), and it therefore cannot be excluded that the characteristic is affected by this. The artifact thus introduced is though probably small. At lengths below $L_0$, where the artifact would be expected to produce the greatest effect, our results are, as described above, similar to those previously reported for vascular smooth muscle. We therefore believe that at lengths above $L_0$, where we have provided new information, the possible error caused by this artifact is very small.
Cell Mechanics

The relation of the active and passive tension-length characteristic of whole smooth muscle to the corresponding characteristics for the individual smooth muscle cells has not been clear. We have here established (Fig. 3) that above $L_0$, at least, changes in vessel internal circumference produce on average the same percentage changes in cell length. This is in agreement with our previous findings (Halpern et al., 1978) and with the results of an indirect technique reported by Murphy et al., 1977. However, our observation that this relationship is not exact for individual cells, and also that activation produces a substantial rearrangement of cells, with some lengthening and others shortening, makes it probable (Herlihy and Murphy, 1973) that not all cells are at the same point on their active tension-length characteristics. Therefore, the curve that we have determined for the preparation can only be taken as an expression of the average characteristics of the smooth muscle cells within it. Thus, there is no reason for supposing that our results necessarily conflict with the preliminary findings of Fay (1975) concerning the active tension-length relationship of his isolated smooth muscle preparation. His curve suggests that the working range of the cells may be even greater than that of our preparation.

Fay (1975) also showed that the resistance to stretch of the relaxed cells was very small even at lengths up to three times their resting length. On this basis the passive tensions we have measured must arise almost entirely from the extracellular connective tissue, which consists mainly of collagen and elastin (Dobrin, 1978). In other words, the active and passive characteristics appear to arise from two independent but parallel structures: the smooth muscle cells and the extracellular matrix, respectively.

Dynamic Responses

The determinations of the active and passive isometric release responses (IRRs) show that the relation between active dynamic stiffness and active tension is remarkably similar to that found in skeletal muscle. Below $L_0$ active dynamic stiffness falls more slowly than active tension, but above $L_0$ the ratio between the two parameters is constant (Bressler and Clinch, 1974, 1975). Huxley and Simmons (1972) have examined the IRR of skeletal muscle fibers at $L_0$ and $1.5L_0$ and show not only the same proportionality between active tension and active dynamic stiffness, but also that the time-course of the responses is similar at the two lengths. This is certainly similar to our observations concerning the time-course of the IRR above $L_0$. Bressler and Clinch and Huxley and Simmons both attribute their findings primarily to the mechanics of the cross bridges between the contractile filaments, by explaining the changes at greater lengths in terms of decreased overlap between the contractile filaments. In our studies, although the IRR can in part be attributed to the active structures within the vessel (Mulvany, 1979), its form appears to be substantially influenced by the passive structures in series with the contractile apparatus. Therefore, despite the similarities of the properties we have found to those of skeletal muscle, it is unlikely that our findings can also be interpreted directly in terms of crossbridge mechanics.
Meiss (1978) has presented very similar results for the relation between active
dynamic stiffness and active tension below $L_0$ in rabbit mesotubarium, and has
attributed it to an increased intracellular resistance at the shorter lengths.
Although we feel that this is an interesting possibility, our histological evidence
indicates that at the short internal circumferences, the cell arrangement becomes
extremely irregular, so that we feel that in our preparation it would be unwise
to draw any definite conclusions.

Relation to Cell Ultrastructure

In skeletal muscle the active tension-length relation has been fully explained in
structural terms. The relationship is similar to that of the individual muscle
fibers and, particularly at lengths greater than $L_0$, the tension is proportional to
filament overlap (Julian et al., 1978). Although, as described in the Introduc-
tion, there is now good structural evidence that the contractile proteins of
smooth muscle are arranged in filaments, the heterogenous nature of smooth
muscle morphology and ultrastructure has so far prevented the nature of the
filament interaction from being established. It is therefore not possible at
present to explain the active tension-length relation of smooth muscle in
structural terms. Two of our findings, however, support the evidence that the
contractile filaments of smooth muscle are at least held in structured arrays
(Ashton et al., 1975; Small, 1977) rather than consisting of a suspension of free-
floating filaments. First, we have found that below 1.4 $L_0$ the active tension is
almost entirely a function of muscle length and not the protocol to which it has
been subjected previously. Second, the failure of the vessel to recover tension
following activation at higher degrees of extension suggests that, because we see
no signs of cellular destruction, it may be irreversible changes in the relationship
of the contractile filaments which are caused by such extension. One attractive
possibility is that the “sarcomere” lengths of smooth muscle are not uniform but
distributed. Lengthening could then result in just the longer sarcomeres being
pulled out of register (and thus irreversibly damaged), so that on release it is
just the remaining sarcomeres which can develop tension. However, the detailed
correlation of the active tension-length relation with structure must await the
results of further structural investigations.

APPENDIX

Characteristics of Isometric Release Responses

The active and passive dynamic characteristics of vessels have been investigated by
determining their response in activating and relaxing solutions to 5-ms $\sim$0.01 $L_0$ releases
(Fig. 2). Such releases were performed over the whole range of internal circumferences
investigated, so that above $L_0$ the isometric release responses (IRRs) in activating solution
were greatly influenced by the passive properties of the preparation. We have therefore
for each internal circumference determined the passive IRR (that is, the mean IRR in
relaxing solution before and after activation) and the active IRR, which is defined as the
difference between the IRR in activating solution and the passive IRR. These calculations
were performed using a mini-computer (Fig. 1) which initiated the releases, measured

the outputs of the force and displacement transducers on-line, and stored the measurements both for immediate analysis and for later more detailed assessment. The acquired data and the calculated active and passive IRRs for the response shown in Fig. 2b are shown in Fig. 9.

The measurement sampling interval was 1 ms for the first 24 ms following the release, after which the sampling time was increased in a geometric progression, such that the response was sampled 51 times up to 10 s. The method of computing the active and passive IRRs was as follows. We denote the tension measurement \( T(t, x) \), where \( t \) is the sample time (that is, the time after the initiation of the release in seconds at which the measurement was made), and \( x \) denotes whether the measurements were made during the initial period in relaxing solution \((x = 1)\), the period in activating solution \((x = 2)\), or the final period in relaxing solution \((x = 3)\). From the measurements in relaxing solution the time-course of the passive IRR, \( T_p(t) \), was calculated for each sample time from:

\[
T_p(t) = \frac{T(t, 1) + T(t, 3)}{2}.
\]

The active IRR, \( \Delta T(t) \), was calculated for each sample time from:

\[
\Delta T(t) = T(t, 2) - T_p(t).
\]

The magnitude of the step change in pusher displacement, \( \delta s \), was set at the start of each experiment so that \( \delta s = 0.005 L_0 \). Note that because a displacement step of \( \delta s \) produces a step change in internal circumference of \( 2 \cdot \delta s \), this corresponds to a change in internal circumference of \( 2L = 0.01 L_0 \). The output of the displacement transducer was also measured by the computer so that in each release the magnitude of the step was controlled.

The characteristics of the IRRs were described by calculating the following parameters:

(a) Passive and active initial rate constant of tension recovery, \( r_p \) and \( \Delta r \), defined, respectively, by

\[
r_p = \frac{\Delta T_p(0.005)}{[T_p(0.0) - T_p(0.005)]},
\]

and

\[
\Delta r = \frac{\Delta T(0.005)}{[\Delta T(0.0) - \Delta T(0.005)]}.
\]
where \( \dot{T}_p(0.005) \) and \( \Delta \dot{T}(0.005) \) are the rates of change of \( T_p \) and \( \Delta T \) at time \( t = 0.005 \) s;

(b) Passive and active recovery at 10 s, \( \eta_p \) and \( \Delta \eta \), defined respectively, by

\[
\eta_p = \frac{T_p(10.0) - T_p(0.005)}{T_p(0.0) - T_p(0.005)},
\]

and

\[
\Delta \eta = \frac{\Delta T(10.0) - \Delta T(0.005)}{\Delta T(0.0) - \Delta T(0.005)}.
\]

(c) Passive and active dynamic stiffness, \( k_p \) and \( \Delta k \), respectively, by

\[
k_p = \frac{\Delta T(0.0) - \Delta T(0.005)}{8L},
\]

and

\[
\Delta k = \frac{\Delta \dot{T}(0.0) - \Delta \dot{T}(0.005)}{8L}.
\]

In Fig. 8c, \( k_p \) and \( \Delta k \) are presented with reference to tension as \( (k/T)_p \) and \( \Delta (k/T) \), defined respectively by

\[
(k/T)_p = k_p/T_p(0.0),
\]

and

\[
\Delta (k/T) = \Delta k/\Delta T(0.0).
\]

In each case the tension measurements used were obtained by fitting smooth curves to at least seven data points as follows:

- \( T_p(0.0), \Delta T(0.0) \) men of seven values obtained at 1-ms intervals immediately prior to the release.
- \( T_p(0.005), \Delta T(0.005) \) obtained from a logarithmic curve of form \( T = A + B \cdot \log(t) \), where \( A \) and \( B \) are constants, (Mulvany and Halpern, 1976) fitted to nine tension values obtained at 1-ms intervals immediately subsequent to the completion of the release from \( t = 0.005 \) s to \( t = 0.014 \) s. Coefficient of determination, \( r^2 \), of fit is normally about 0.9.
- \( \dot{T}_p(0.005), \Delta \dot{T}(0.005) \) slope of above curve at \( t = 0.005 \) s
- \( T_p(10.0), \Delta T(10.0) \) obtained from logarithmic curve of same form as above, fitted to the final seven tension values between \( t = 2 \) s and \( t = 10 \) s. \( r^2 \) is normally at least 0.99.

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