Effect of stretch on passive tension and contractility of isolated vascular smooth muscle

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SPARKS, HARVEY V., JR., AND DAVID F. BOHR. Effect of stretch on passive tension and contractility of isolated vascular smooth muscle. Am. J. Physiol. 202(5) : 835-840. 1962.—Helically cut strips of the wall of small branches of dog superior mesenteric artery were stretched in a stepwise fashion. Tension developed in response to stretch or to a standard stimulus (epinephrine or electricity) was recorded isometrically. The elastic diagram of the vessel is comparable to that reported by other investigators. Contraction in response to a standard stimulus increased with stretch, as much as 100% for a 10% increase in length. The increase in response continued until the strip reached a certain optimal length (variable from strip to strip), after which the response decreased with further stretch. When the strip was released in a stepwise fashion hysteresis was observed. Possible relationships of tension and length at the level of the contractile element are discussed together with ways in which the information presented here may relate to myogenic autoregulation.

It has been known for some time that contractility of skeletal (1) and cardiac (2) muscle increases with stretch over a certain range. Although this phenomenon has also been defined for some types of smooth muscle (3, 4), it has not been described for that of the arterial wall. The effect that stretch and resting tension have on contractility in this tissue bears directly on the currently contested ideas concerning the autoregulation of blood flow in response to changes in perfusion pressure. A change in the distending pressure within a blood vessel will certainly alter the tension and length of the muscle fibers of the vessel wall. Since in vivo the musculature of the resistance vessels is partially contracted (5), the effect that such changes in length and resting tension have on the contractility of the vascular smooth muscle will have a direct bearing on the amount of active tension in the vessel wall and therefore on a possible mechanism of autoregulation.

Since the original postulate of Biedl and Reiner (6) and later of Bayliss (7) attributing autoregulation to a myogenic response produced by an increase in distending pressure, this explanation has been extensively supported (8-10) and refuted (11, 12), always by the indirect method of pressure-flow studies. In the light of these contradictory ideas concerning the mechanism and even the existence of autoregulation, the current study was undertaken to define the effect of stretch on the resting tension and contractility of isolated vascular smooth muscle.

METHODS AND MATERIALS

Radicles of dog superior mesenteric artery were chosen as the source for the vascular smooth muscle because of their predominantly muscular media. The mesentery supporting the lower jejunum and ileum was removed and stored in Krebs solution (composition in mm/liter: NaCl, 118.9; KCl, 4.7; NaHCO₃, 14.6; MgSO₄, 1.2; KH₂PO₄, 1.2; CaCl₂, 2.5; sucrose, 49.9; dextrose, 5.5; and calcium disodium versenate, 0.026) at 4°C until used (not more than 4 days). No change in contractile characteristics or elastic properties of the vessels was associated with length of storage within this period. An arterial segment with an inside diameter of approximately 1 mm was taken from the mesentery, stripped of loose fat and adventitia, and the wall was cut helically to obtain a strip 10-25 mm long, 0.8-1 mm wide, and approximately 0.2 mm thick. Each end of the strip was tied to a platinum hook, and it was mounted in a bath of Krebs solution aerated with a mixture of 95% O₂ and 5% CO₂ at 38°C. The lower platinum hook was attached firmly to a stationary glass rod; the upper hook was extended to a force transducer (Grass model FT 03) whose electrical activity, representing tension of the strip, was recorded by a pen-writing oscillograph. The transducer was mounted on a movable support which permitted adjustment of the length of the strip to within 0.2 mm.

The muscle was allowed to equilibrate, hanging loosely in the bath, for 2 hr to permit correction of abnormal electrolyte conditions resulting from storage...
and the trauma of mounting. The transducer support to which the strip was attached was then adjusted until the first increase in tension was recorded. The length of the strip at this point was designated the "initial length," and the base line recorded at this length was considered to represent 0 g tension. Stretch increments employed were from 6 to 25% of the initial strip length; they were effected by adjustment of the transducer support (this required 3-5 sec) and were spaced at 20-min intervals. Stretch adjustments were continued until the strip was approximately doubled in length, at which time the process was reversed and the muscle length was decreased at 20-min intervals in decrements comparable to the previous increments.

When the effect of stretch on the magnitude of the response was to be studied the standard stimulus was an electric current or epinephrine. Sixty-cycle current for electrical stimulation was supplied through an a-c rheostat and monitored by observing the voltage drop across a 1,000-ohm resistor in series with the strip. The voltage across the length of the strip varied from 5 to 12 v among the strips but was kept constant for any one strip. The platinum hooks served as electrodes. The bath was lowered away from the strip during the 5-set stimulation period. Where a humoral stimulus was used, epinephrine was added to the bath to give a concentration of 10^-7 g/ml of bath solution. The epinephrine was removed by flushing the chamber several times with warm aerated Krebs until the tension of the strip reached its prestimulus resting value. The stimulus, electrical or humoral, was applied 10 min after length adjustment, therefore at 20-min intervals.

RESULTS

Stretch-passive tension relationship. A record of tension changes in response to a single stretch increment (Fig. 1) shows an initial abrupt increase in tension followed immediately by a gradual fall to a steady state higher than the tension before the strip was stretched. The new tension level was usually reached within 10 min. An elastic diagram has been prepared by plotting steady state tension values after successive stretch increments against the strip length after the stretch. Such an elastic diagram is presented in Fig. 2 (solid line). The amount of tension increase after stretching is greater as the strip is longer; after the strip has been stretched more than 50% of its initial length the tension rise is steep. Steady state tension values accompanying a series of decrements in strip length (Fig. 2) decreased sharply, and zero tension was reached before the strip returned to its initial length, giving evidence of hysteresis.

Stretch-active tension relationship. The effect of stretch on the magnitude of the tension developed in response to a standard submaximal electrical stimulus also is shown in Fig. 2 (dashed line). (A submaximal stimulus was used because the muscle cannot tolerate at maximum strength the repeated stimulation required by this study.) With each increase in length there is a corresponding increase in magnitude of the response until an optimal length is reached, with further increases in length there is progressive decrease in response. Generally similar results were obtained with the ten artery strips tested. There were, however, real quantitative differences among the strips in a) maximum contractility expressed either in absolute values or as per cent increase over active tension developed at the unstretched length and b) per cent increase in length at which maximum contractility was observed (Table 1). Possible determinants of this variability will be dealt with under discussion.

Similar studies were carried out using epinephrine as the stimulus. In Fig. 3 is a typical record showing increased response with each successive stretch until the muscle length was more than doubled (125%) after which the response was decreased. This is entirely similar to the effect of stretch on response to electrical stimulation.

Hysteresis was evident in response to stimuli under
stretch, as it was in response to stretch per se. When the strip was released much in the same way it had been stretched, contractility at any given length was never as great as when the strip was being stretched (Fig. 2).

Control studies were made to determine the influence on contractility of the length of time the strip had remained in the bath or the number of times it had been stimulated. The strip was stimulated three times at 20-min intervals, then stretched, and stimulated three more times at 30-min intervals. This was carried out over the same range as the above procedure. Typical results are shown in Fig. 4. They indicate that while there is some variation in contractility at any given length, there is no progressive increase with time or number of previous stimulations, as there is with stretch.

Results from monitoring the stimulating current are shown in Fig. 5. The amount of current passing through the strip is inversely related to the strip length.

Resting tension-active tension relationships. Certain observations made in the course of the current study have bearing on a possible relationship between resting tension and active tension. In one procedure the strip was stretched, then stimulated within 10 sec. The resting tension was then allowed to return to a steady value, and (after approximately 20 min) another stimulus was given. It was found that immediately after stretch, less active tension was elicited in response to a standard stimulus than was elicited by a similar stimulus when the resting tension had fallen to its steady value. This is depicted in the response curves of Fig. 6. The total tension (i.e., active plus resting tension) developed by the second contraction, at 20 min after stretch, was usually equal to or slightly less than that developed by the first contraction.

It was observed in isolated cases that no measurable increase in resting tension occurred when the strip was stretched, yet contractility was increased over that associated with the previous strip length.

### Table 1. Optimal lengths*, absolute values for active tension, and percentage values for active tension, for ten strips

<table>
<thead>
<tr>
<th>STRIP #</th>
<th>OPTIMAL LENGTH IN % INCREASE ABOVE INITIAL LENGTH</th>
<th>ACTIVE TENSION AT OPTIMAL LENGTH, IN MGMS</th>
<th>ACTIVE TENSION AT OPTIMAL LENGTH, IN % INCREASE ABOVE TENSION DEVELOPED AT INITIAL LENGTH</th>
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* Lengths at which greatest active tension response was seen.

#### Discussion

For the purposes of this paper passive tension is defined as a visco-elastic force which develops in response to stretch. It is chronically maintained and reflects the passive physical properties of both the contractile and noncontractile components of the tissue. Active tension is defined as force which develops in response to a stimulus. It is dependent on an ill-defined chemo-mechanical system in the contractile elements of the tissue.

**Passive tension.** Passive tension defined in this way has been considered in two different contexts in recent literature. Burton (13) has used a static elastic diagram of the vessel wall to illustrate the relation between passive tension in the wall and the radius of the vessel. Tension in the wall was calculated by applying La Place's law to measurements of the distending pressure and the volume (and therefore the radius) of the vessel segment. Burton's elastic diagram showing an initial gradual and late steep ascent of the tension axis is similar to that obtained from the direct observations made in the current study (Fig. 2). Peterson (14) and others (15), on the other hand, have pointed out the role of viscosity as an important determinant of wall tension or stiffness in dynamic states. The dynamic state involved is the acute variation in pressure occurring with the pressure pulse. The viscous characteristic of the blood vessel wall is related to the dynamic function of large vessels as a reservoir for blood ejected during systole. The time axes used in the current study were not adequate to evaluate these acute viscous properties of the vessel wall. Furthermore, it seems reasonable that the small, 1-mm mesenteric artery used in the current study does not have as its primary function that of being a dynamic blood reservoir during systole. From its heavy muscular media it can be inferred that it serves more in the function of control of resistance to flow. The slow decrease in passive tension with time after stretch (Fig. 1) may be analogous to the "delayed compliance" of the splanchic vascular bed after a rise in perfusing pressure (16).

**Active tension.** The relationship between active tension
and stretch observed here in the strip of arterial smooth muscle (Fig. 2) parallels that previously reported for skeletal (1) and cardiac muscle (2, 17). The hysteresis loop for active tension (Fig. 2) is similar to that for dog retractor penis (3) and the reverse of that for dog papillary muscle (2). The present observation that response to such dissimilar stimuli as epinephrine and electric current are similarly influenced by stretch strongly suggests that the mechanism of the influence is a basic component of the contractile machine shared by responses to all active agents.

Considerable variability was noted in the magnitude of the increase in active tension development with stretch and in the per cent increase in length at which maximum tension occurred (Table 1). These quantitative variabilities, however, should not obscure the consistency of the positive correlation between stretch and active tension development. The maximum strength of contraction per unit cross-sectional area of strip reflects biologic variation that must occur among dogs (e.g., related age or blood pressure).

Variability in per cent increase in active tension and in optimal length may result in part from the method used for determining initial strip length. “Initial length” is defined as the length at which further stretching of the strip first causes a measurable increase in resting tension. This increase depends not only on the contractile elements of the muscle but also on the passive elastic structures parallel to or in series with them. Biological differences in the amount or constitution of this passive component would cause differences in the initial length unrelated to the condition of the contractile element. Since a 10% increase above initial length causes approximately a 100% increase in response (Fig. 2), a small inaccuracy in initial length determination or slight variability in the passive component will make considerable difference in the total per cent increase in contractility. Any degree of active tension of the contractile element would also alter the initial length and further the variability of this determination, but previous observations indicate that under the conditions of this study the smooth muscle is completely relaxed. Day-to-day variations in technique could affect current density (stimulus) and thus be a source of quantitative variability in the response seen. Operative factors considered in this regard are the length and cross-sectional area of the strip in relation to the voltage used and contact between the strip and the electrodes.

Since current density for a given current increases with decreased cross-sectional area, the possibility that the increased contractility might be due to increased stimulus must be considered. However, measurements of current flow through the strip were reassuring. Doubling the length of the strip (voltage remaining constant) reduced the current flow to 10% (Fig. 5). This implied increase in resistance with stretch is the product of the increase in length and the decrease in cross-sectional area of the strip. Since this increase in length probably reduced the cross-sectional area by no more than half, the current density and therefore the strength of stimulus at this new length must have been considerably reduced.

The problem of identifying length or tension as the determinant of the increased contractility in cardiac muscle has been extensively considered (17–20). Some of the evidence for length as the determinant is that in many different systems the volume of a ventricle has been increased without an accompanying measurable
increase in tension or pressure. An analogous situation, with length substituted for volume, was seen in our experiments. Figure 6 gives evidence that may be interpreted as excluding passive tension as the significant determinant of active tension development since there is an inverse relationship between passive tension and active tension developed, without a change in length. This, however, may merely reflect the possibility that the first of these two responses was obtained at a tension greater than optimal. Further argument that length rather than tension is the significant determinant of active tension development is found in the 100% increase in response observed with the first 10% increase in length, without a recorded change in passive tension (Fig. 2). However, it does not seem justifiable to conclude that, because an instrument records zero tension change, the contractile element "sensed" zero tension change. Bulbring (18) reports that tension may be the determining factor in guinea pig taenia coli because tension is related to the frequency of spike potentials and membrane potential in a more predictable way than is length when the strip is stretched and released. Absence of action potentials of vascular smooth muscle in the contracted state (21, 22) forms a basis for questioning the state of the membrane as a determinant of the contractility-stretch relationship. On the other hand, the contractile elements of glycerol-extracted cardiac muscle contract more vigorously when stretched (23). If we assume that these elements are similar in vascular smooth muscle, this observation would shift the locus for the action of stretch from the membrane to the contractile protein.

The fact that active tension developed in response to a stimulus given immediately after stretch is not as great as that to the same stimulus given later has been reported for dog retractor penis (3). A simple mechanical model (Fig. 7) illustrates some possible relationships between length and tension. If the elastic element in series is the more viscous the immediate effect of stretch might be to pull out the contractile element beyond a good functioning length (Fig. 7A). A stimulus given at this time would elicit a smaller contraction than the same stimulus given later, after the elastic element had stretched and allowed the contractile element to return to a better functioning length (Fig. 7B). This explanation would inerminate tension as the cause of the increased response.

If the contractile element is the more viscous and lengthens very little with stretch while the series elastic component lengthens a great deal (Fig. 7C), then, as the contractile element slowly is pulled out (Fig. 7D), the potentiating effect of increased length will become obvious and length becomes the determining factor.

How does the in vitro information presented here relate to the in vivo process of myogenic autoregulation? When pressure increases in the lumen of a small artery, the tension in the wall increases also. Most data which reflect the process of autoregulation seem to indicate that there is a period after the increase in pressure during which the wall stretches in response to the increased tension and the flow increases; subsequently the vessel contracts and the flow diminishes in spite of the greater pressure (7, 8, 10, 24).

Since in vivo the musculature of arteries is constantly partially contracted due to sympathetic tone and myogenic basal tone (5), a stretch of the muscle caused by increased pressure in the lumen would cause increased contractility (Fig. 2) and thus increase the total tension in the wall. Since the diameter of the vessel is determined by the equilibrium between the tension in the wall and the pressure in the lumen, the greater active tension elicited by stretch would result in decrease in the caliber of the vessel at the elevated perfusion pressure. In this way, an increase in perfusion pressure would result in a less than proportional increase in flow.

An interesting feature of autoregulation is that it requires that the increase in the transmural pressure cause the vessel to constrict to a circumference smaller than it was before the increase in pressure was applied. This eliminates the possibility that the process is maintained by lengthening of the over-all circumference. In order to account for the maintenance of such a myogenic response, it is necessary to visualize an elastic element in series with the smooth muscle cells which, by imposing increased tension on the individual cells, causes these to shorten so that the over-all circumference is less than it was before the tension in the wall was increased. Observations in the current study do not permit an analysis of the role of this hypothetical series elastic element.
ADDENDUM

Since this paper was written we have become aware of pertinent work done on this subject by N. R. Speden (J. Physiol., London 154: 15, 1960). We find that the results of our study, using dogs, are in accord with those of Speden for sheep; the conclusions of both studies are thereby fortified.

REFERENCES