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Velocity and myosin phosphorylation transients in arterial smooth muscle: effects of agonist diffusion[1]

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Summary. Transients in myoplasmic [Ca^{2+}] and in phosphorylation of the 20,000 dalton light chain of myosin have been reported following stimulation of vascular smooth muscle by various agonists. Since these transients are rapid compared with the time required to attain a steady-state stress, agonist diffusion rates may be a significant limitation in activation. The purpose of this study was to estimate the effect of agonist diffusion rates on the time course of activation as assessed by mechanical measurements of stress development and isotonic shortening velocities and by determinations of the time course of myosin phosphorylation. The approach was to measure these parameters in K^+-stimulated preparations of the swine carotid media of varying thicknesses and to estimate the theoretical contributions imposed by diffusion rates and the presence of a diffusion boundary layer surrounding the tissue. The results show that the time course of parameters which are tissue averages such as stiffness, active stress, and myosin phosphorylation is dominated by agonist diffusion rates. The sequence of events involved in excitation-contraction coupling including agonist actions on the cell membrane, Ca^{2+} release, activation of myosin light chain kinase, and cross-bridge phosphorylation appear to be very rapid events compared with stress development. Estimates of unloaded or lightly loaded shortening velocities which are not simple tissue averages appear to provide an improved estimate of activation rates.
Key words. Myoplasmic Ca^{2+}; smooth muscle mechanics; active state; potassium depolarization; myosin light chain; swine carotid artery.

Introduction

The kinetics of activation for mammalian smooth muscle tissues in vitro are a function of the rates of several processes: 1) agonist diffusion and action on the cell membrane; 2) an increase in myoplasmic [Ca^{2+}] following release from intracellular stores or influx through the plasma membrane; 3) cross-bridge activation which appears to be a consequence of Ca^{2+}-calmodulin-induced activation of myosin light chain kinase and phosphorylation of the 20,000 dalton myosin light chain[7,18,24]; and 4) cross-bridge cycling resulting in stress development or shortening.

Stimulation of intact smooth muscle strips is associated with increased phosphorylation of myosin, maximal values of which are reached in less than one minute. The initial peak levels of phosphorylation usually decline in time, while active stress rises monotonically to a maintained steady state[6,10,17,33]. It has been proposed that agonist-induced transients in cell calcium concentration[29] can explain a rapid, large phosphorylation transient in the smooth muscle cell[3].

Estimates of myosin light chain phosphorylation are tissue averages. A rapid phosphorylation transient in cells on the edge of the tissue may be virtually complete before the inwardly diffusing agonist reaches excitatory concentrations in the center of the strip. The purpose of the present study was to evaluate the contribution of agonist diffusion times to estimates of rates and magnitudes of activation measured in isolated tissues. The results indicate that 1) the onset of phosphorylation in vascular smooth muscle cells is a very rapid event following stimulation; and 2) that the early time course of parameters such as myosin phophorylation and stress development, which are tissue averages, will be dependent upon agonist diffusion times.

Methods

1. Solutions

The standard physiological salt solution (PSS) used in these studies contained (mM): NaCl, 140.1; KCl, 4.7; Na_2HPO_4, 1.2; $MgSO_4$, 1.2; $CaCl_2$, 1.6; D-glucose, 5.6;

morpholinopropane sulfonic acid (MOPS) buffer (pH 7.4 at 37 °C), 2.0; and ethylenediamine N,N′-tetraacetic acid (EDTA), 0.02. The 109.7 mM high potassium solution (K^+-PSS) had KCl substituted for NaCl to maintain isosmolarity. Solutions were maintained at 37 °C and gassed with 100% oxygen.

2. Tissue preparations

Swine arteries were collected, cleaned of loose adventitial tissue, and stored in PSS at 4 °C overnight before use[11]. Tissues prepared from the swine carotid media have several important characteristics for the experimental studies. 1) The rectangular cross-section is uniform, the cells are aligned in the axis of force measurement[20]. 2) Extensive studies have shown that values of active stress (N/m^2) and shortening velocity (optimum muscle lengths (L_o)/sec) obtained from the tissue provide accurate estimates of cellular mechanical output under steady-state conditions[13]. 3) The tissues have a high viability, as estimated by the ability to generate very high active stresses (up to 6.7×10^5 N/m^2 of cell cross-sectional area) throughout lengthy experiments[11]. 4) Furthermore, their anatomy, consisting of 22 parallel elastic lamina separating muscle layers two to three cells thick[34] enabled us to prepare strips of different thickness with minimal tissue damage.

Media tissues were dissected as illustrated by Driska et al.[14]. The procedure was slightly modified for thin strips (i.e., tissues in which not only the adventitia was removed, but also layers of muscle from the intimal side). The intimal layers were dissected away after the media was partially separated into two layers with a razor blade. The remaining media was cut to a width of 1.5 to 2.0 mm after removal of the adventitial sheath (lower diagram, fig. 1B, see Driska et al.[14]. Viable strips with thicknesses as low as 180 µm could be obtained which met the following criteria. 1) The strip should develop values of active stress approaching those of full thickness strips obtained without the additional dissection (minimal tissue damage). 2) The strips had to maintain full responsiveness for periods up to 20 h. 3) The strips had to be large enough (≥ 2 mg) for light chain phosphorylation measurements. 4) Strip thickness had to be uniform.

Obtaining accurate estimates of tissue thickness was critical. Previous studies using direct optical measurements of the rectangular carotid tissues with a Filar micrometer mounted in the eyepiece of a dissecting microscope[20] showed that cross-sections could be accurately calculated from measurements of wet weight of lightly blotted tissues and length (L_o), assuming a density of 1.05 g/cm^3. Subsequent studies showed that errors due to variations in hydration and/or blotting procedures were negligible, as similar cross-sections were calculated using tissue dry weights after correction for tissue water content[11]. Two additional methods were used in the present study to estimate tissue thickness and cross-sectional area and to compare the values obtained with those calculated from wet weight. One method involved a direct measurement of thickness at the center of a tissue mounted at its optimal length (L_o) using a vernier caliper. The other involved microscopic measurements on cross-sections of tissues fixed at L_o with neutral buffered isosmolar formalin. Each method is subject to specific types of errors, but the four procedures explored in this laboratory all gave values falling near the identity line when plotted against thickness calculated from wet weight, length and width at L_o, and density. The latter procedure was routinely used in subsequent work.

Tissues were mounted in jacketed tissue baths between a rigid support attached to a micrometer and Grass FT.03C force transducers for isometric studies or to an electronic lever apparatus for isotonic experiments. After equilibration at 37 °C in oxygenated PSS for one hour, partial length-stress curves were obtained by the method of Herlihy and Murphy[20]. The length was then adjusted to the optimal length (L_o) for maximal stress development (S_o).

3. Servosystem

Isotonic shortening velocity measurements were made with an electronic lever system[12]. Isotonic quick-releases to a preset afterload were made at a given time after the initiation of stimulation by replacing the PSS with PSS containing the agonist. The release was made by manual switching of the current to the lever armature to a new value which resulted in a specified load against which the muscle shortened. Values of isotonic shortening velocity were determined using a linear regression program, which estimates dL/dt at the moment of release from the slope of the natural logarithm of the normalized length vs. time. Length values at times between 1.0 and 2.2 sec after release when the muscle was shortening at a constant load were used for the calculation of velocity[12]. Loads were chosen as a fraction of the maximum active stress developed in response to a specific stimulus condition. Velocity measurements after 60 sec in K^+-PSS were made repeatedly on one muscle strip to estimate the error of measurement. Under these conditions, the velocity at a load equal to 10% of the maximum stress induced by K^+-PSS ($V_{0.1\,S_o}$) averaged 0.0299 ± 0.0017 L_o/sec ($\bar{x} \pm$ SD, $N = 6$, $SD/\bar{x} = 0.058$). The lever system, including its performance characteristics and calibration procedures are described by Dillon et al.[12].

4. Phosphorylation measurements

Tissues mounted for isometric studies were frozen by immersion in a dry ice/acetone slurry (−78 °C)[14] after specified times of stimulation. The brief stimulation periods involved in determining the initial phosphorylation in this study required careful timing. This was facilitated by replacing the jacketed bath with a beaker containing 250 ml of K^+-PSS (rapidly bubbled with oxygen, 37 °C) at time = 0 to start the contraction. This beaker could be replaced with another containing the freezing mixture within 0.5 sec. The center of the thickest strip studied (550 µm) should reach −15 °C in 0.12 sec according to calculations using a value of thermal diffusivity of 0.0021 cm^2/sec[31]. The total denatured tissue homogenate was subjected to two-dimensional gel electrophoresis[14]. The relative amounts of separated phosphorylated and nonphosphorylated light chains were measured by scanning the appropriate region of the Coomassie Brilliant Blue stained SDS-slab gel. The fraction of the phosphorylated light chain was calculated relative to the sum of the phosphorylated and nonphosphorylated 20,000 dalton light chains. This technique requires rigid adherence to the protocols[3].

5. Statistics

Data are reported as means±SEM unless otherwise

noted. Regression lines were obtained using the method of least squares. Statistical comparisons were made by use of Student's t-test and assumed significant at $p < 0.05$.

Results

1. Effect of strip thickness on mechanical responses

The most direct approach to estimating the effects of agonist diffusion time on rates of activation in tissues is to vary the length of the diffusion path and determine the effects on the time courses of processes involved. High K^+ was selected as the agonist as this ion has a high diffusion coefficient in tissues, is not metabolized, and the concentration giving maximal activation (60 mM)[32] can be rapidly attained in the center of the tissue using 110 mM K^+-PSS. Strips of varying thickness were dissected and time courses of stress development, changes in isotonic shortening velocities and in light chain phosphorylation were measured. Thin strips averaging 268 ± 15 µm in thickness developed active stresses of $2.21 \pm 0.33 \times 10^5$ N/m^2 ($n = 9$) in respone to K^+-PSS, or approximately 80% of the 2.7×10^5 N/m^2 active stress generated by the standard full thickness media preparation. In general, values of S_o decreased with tissue thickness, which we attribute to the increasing contribution of edge effects due to superficial cell damage. Shortening velocities after release to a load of 0.1 S_o were comparable in thin and thick tissues in steady-state contractions (0.015 and 0.016 L_o/sec, respectively).

A significant diffusion limitation is presented by the unstirred layer surrounding the tissue. While its thickness can be reduced by increasing solution mixing rates, the boundary layer cannot be entirely eliminated[9]. Preliminary experiments demonstrated that the rate of rise of stress development in response to K^+ depolarization was slower without aeration or stirring and increased with mixing rates up to a maximum which was readily attained with moderate 0_2 bubbling rates in tissue baths having the sintered glass gas dispersion tube centered below the tissue. Subsequent experiments were performed using higher bubbling rates to reduce the diffusion boundary layer to the lowest practical value. The period of latency between introduction of K^+-PSS into the chamber and initiation of contraction was measured. The precise time of application of stimulus was recorded by measurement of a change in resistance between electrodes placed at the level of the bottom of the strip with non-stimulatory voltages applied. The latent period between stimulus and force development varied from 2.1 to 5.5 sec averaging 4.3 ± 0.9 sec ($n = 6$). An estimate for the unstirred boundary layer of approximately 140 µm can be calculated using a diffusion coefficient of 1.94×10^{-5} cm^2/sec for K^+ in aqueous solution of 37 °C[16] and assuming edge concentrations must rise to 60 mM after 4.3 sec for initiation of force development.

Figure 1 shows the time courses of stress development and shortening velocity after release to a constant afterload (0.1 S_o) for a full thickness media preparation and a thin strip after stimulation with K^+-PSS. Active stress was normalized to the maximal stress for each strip to illustrate the more rapid rate of tension development in the thin strip (the times to half-maximal stress were $t_{0.5\ s_o} = 15$ sec and 25 sec in the thin and thick tissues, respectively). Isotonic releases were made after the tissue was exposed to high potassium for a given period. Peak velocity was estimated using measurements obtained at 2.5 sec intervals.

In the thick media preparations, velocity rose to a broad peak between 20 and 50 sec and then fell with time. In thin tissues, the time to peak velocity decreased, peak velocities increased, and the initial fall from peak shortening rates was greater. The time to peak velocity (TPV) was used as an index of the initial rates of activation. For more rounded curves, the TPV was chosen to be the earliest time point at which the shortening velocity was within the measurement precision of the maximum measured velocity (one SD or 6% estimated from repeated measurements). In figure 1 for example, maximum velocity occurred at 25 sec in the 460 µm strip; TPV was 20 sec since the velocity at this time was only 4.4% less than at 25 sec. The TPV obtained by this method could be reproduced in three consecutive time course measurements in an individual tissue.

A plot of TPV as a function of the square of tissue half-thickness (fig. 2) shows that TPV was independent of the diffusion path when strips were less than 420 µm thick. The velocity transient was highly reproducible over this range of strip thickness (210–400 µm). In these prepa-

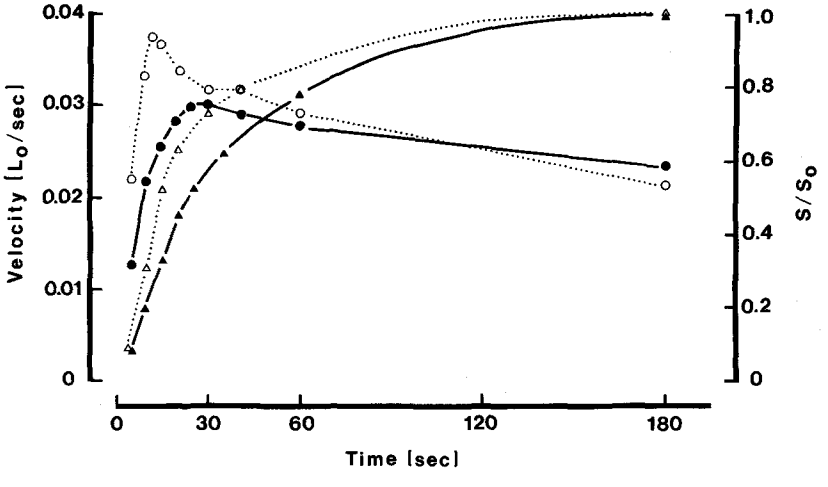

Figure 1. Time course of active stress development (triangles) and of isotonic shortening velocity (circles) at a constant afterload of 0.10 S_o in a full thickness carotid medial tissue (460 µm, *solid symbols*) and a thin tissue (210 µm, *open* symbols). Tissues were exposed to K^+-PSS at 0 sec. Stresses were 2.8×10^5 and 2.1×10^5 N/m^2 in the thick and thin tissues, respectively.

rations, TPV normally fell between 12.5 and 17.5 sec, averaging 14 ± 1 sec.

2. Time course of myosin phosphorylation in thin tissues

Myosin phosphorylation by myosin light chain kinase is a directly measurable estimate of Ca^{2+}-induced activation in smooth muscle. In previous studies of K^+-stimulated tissues[4,10], we found that myosin phosphorylation was always correlated with values of V_o or with $V_{0.1\,S_o}$ under conditions where these parameters were not rapidly changing with time. This correlation should also be observed during the rapid initial transients in thin tissues if 1) phosphorylation is one factor which determines isotonic shortening velocities as we have hypothesized[3] and if 2) both parameters provide equivalent estimates of tissue activation. The data shown in figure 3 suggest that one of these conditions cannot be correct as shortening velocities increased more rapidly than did myosin phosphorylation after stimulation of thin tissues with K^+-PSS. A linear regression line for values of phosphorylation between 0 and 17 sec is plotted. This relation indicates that the level of tissue phosphorylation does not exceed basal values until 4.4 sec after stimulation. This delay is consistent with estimates of potassium diffusion time through an unstirred layer of 140 μm in thickness. We tested the possibility that cooling during the brief period when the bath was lowered to freeze the tissues might have decreased the phosphorylation levels, particularly in the earliest time points. Consequently, the velocity measurements were repeated using a protocol in which the bath was lowered. A significant difference in the rates of activation estimated by velocity and phosphorylation was still observed.

3. Calculation of phosphorylation transients

Figure 4 illustrates calculated potassium concentration profiles across medial strips of two thicknesses. These profiles were calculated from the equations describing diffusion of a substance into a plane sheet:

$$\partial C/\partial t = D\partial^2 C/\partial x^2$$

The initial and boundary conditions are:

$$C = C_0 \qquad -l < x < l \qquad t = 0$$
$$C = C_1 \qquad x = \pm l \qquad t > 0$$

where C_0 is any initial concentration of agonist (normally zero), C_1 is the total concentration of agonist present on stimulation, x is the distance from the surface of the strip, l is the half-thickness of the strip, and t is time. The solution of this equation[8] is:

$$\frac{C - C_0}{C_1 - C_0} = 1 - \frac{4}{\pi} \sum_{n=0}^{\infty} \frac{(-1)^n}{2n+1} \exp \frac{-D(2n+1)^2\pi^2 t}{4l^2} \cos \frac{(2n+1)\pi x}{2l}$$

The K^+ concentration profiles were calculated for experimental conditions in which external $[K^+]$ was raised from 4.7 to 109.7 mM. A value of D equal to 1.1×10^{-5} cm²/sec was taken from estimates of D_{42K} in the extracellular space of dog carotid artery at 37 °C[25]. A step change in external $[K^+]$ at the edge of a strip 250 μm thick, which immediately depolarizes the superficial cells, does not produce maximal stimulatory levels of $[K^+]$ (60 mM)[32] for cells at the center of the strip until 6 sec after the solution

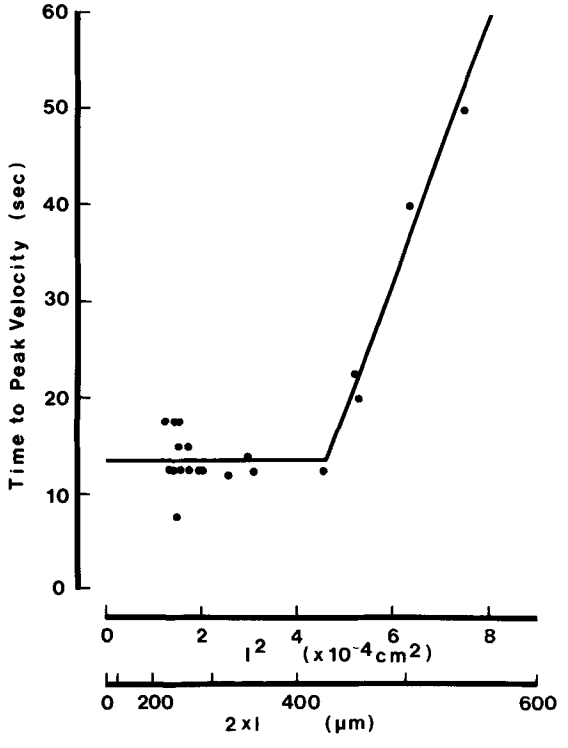

Figure 2. Time to peak velocity after stimulation with K^+-PSS is plotted as a function of the square of one-half strip thickness l. The time resolution of the measurements was 2 to 2.5 sec. The calculated least-squares regression line is shown for values measured at thicknesses greater than 400 μm (TPV = $1.3 \times 10^5\, l^2$–48.5 sec; r = 0.99). Also shown is a horizontal line representing the average TPV for strips less than 400 μm thick. A second abscissa shows strip thickness in micrometers.

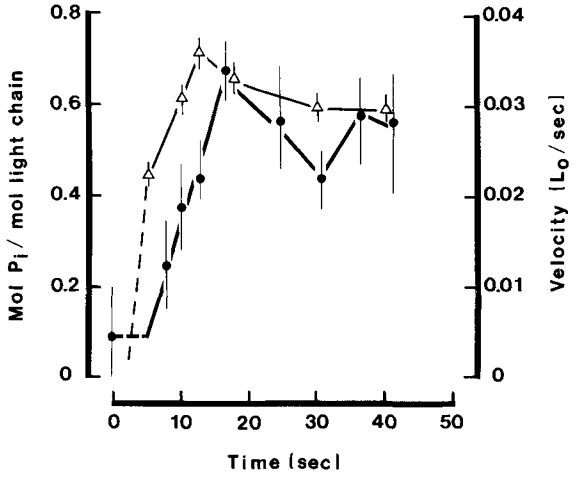

Figure 3. Myosin light chain phosphorylation (●) in thin tissues (190–370 μm) frozen after stimulation by K^+-PSS (averages are plotted with the full range of values indicated by bars, N = 2–6). Values measured between 0 and 17 sec (where peak phosphorylation occurred) were fitted by the method of least squares to a line which is plotted over that range (LC-P = 0.047 t–0.13 mol P/mol LC, r^2 = 0.89, N = 11). The relation predicts that light chain phosphorylation will not exceed 0.08 mol P/mol LC (that is basal levels) at 4.4 sec following stimulation. This is shown by a dashed horizontal line. Average values of shortening velocity (△) at a constant afterload of 0.104 ± 0.002 S_o are shown for thin strips (210–352 μm). Velocity is zero until developed stress is equal to the load of 0.1 S′ (dashed line).

is changed. Figure 4 also shows that the rate of change of agonist concentration is diminished toward the center of the muscle strip, which approximates a sheet. Maximal stimulation of central cells by 60 mM K^+ requires 23 sec in a 500 μm strip. A second diffusional delay will be added by the presence of an unstirred layer of 70–150 μm thickness adjacent to the muscle sheet[19]. The presence of the unstirred layer has two effects on the rate of agonist-induced stimulation. First, there is no longer a step change in agonist concentration at the edge of the muscle strip. Delays of 0.5–5.0 sec to maximal stimulation of edge cells will occur depending on the true thicknesses of the unstirred layer. Second, the times to maximal stimulation at all positions (values of x) across the strip will be prolonged. This analysis shows that agonist diffusion may dominate true cellular time courses for myosin phosphorylation and mechanical activation.

The time course of tension development in response to external stimulation will also be influenced by properties of the muscle tissue itself. Contraction will be accelerated if stimulation of superficial cells is propagated across the media at a more rapid rate than agonist diffusion proceeds[28]. Besides cell coupling, excitation may be propagated by agonist stimulation of nerves causing release of neurotransmitters at more distant sites.

Such considerations rule out a precise theoretical es-

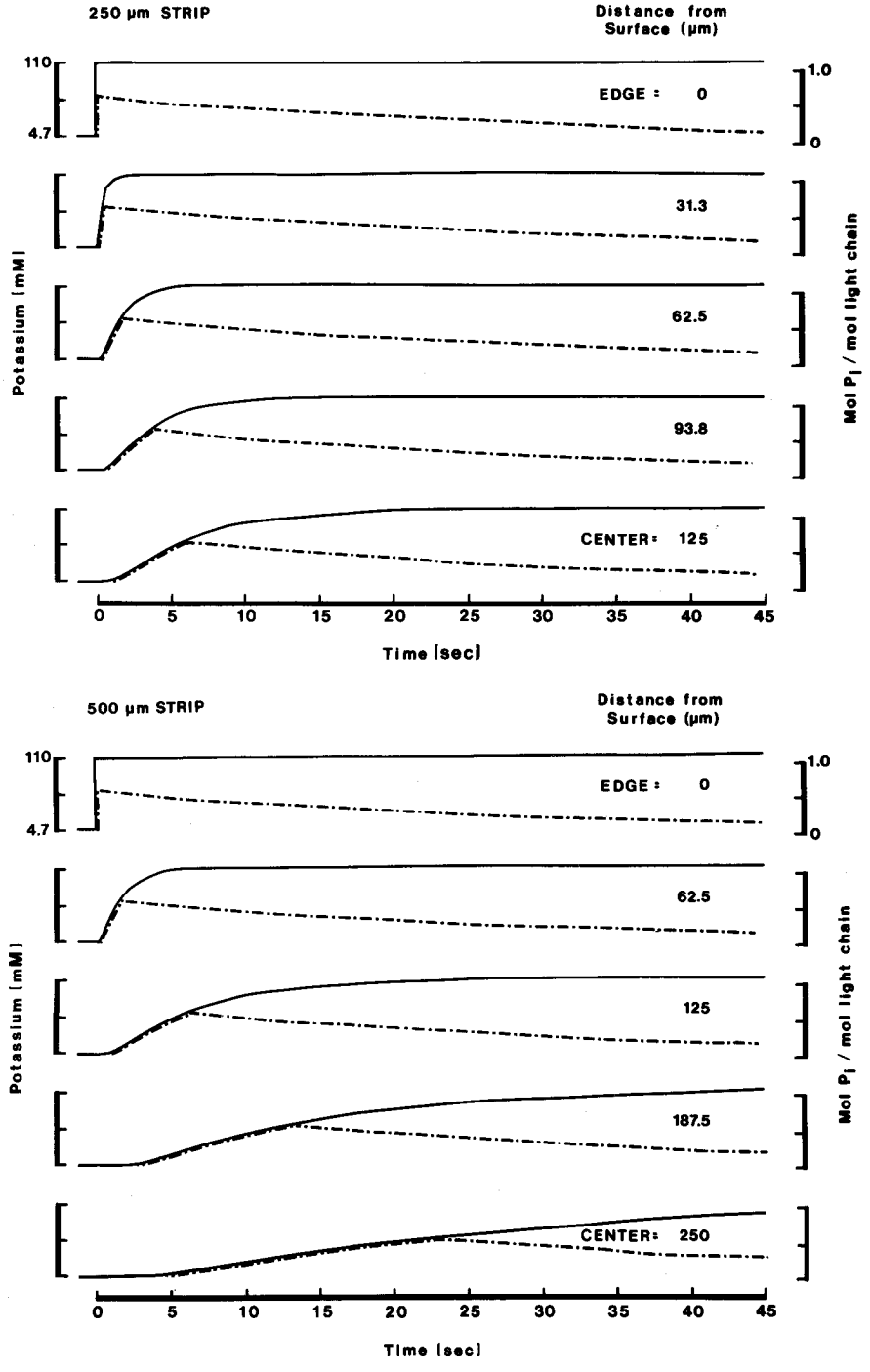

Figure 4. Concentration profiles for potassium at varying distances from the surface of 250 μm (upper panel) and 500 μm (lower panel) thick tissues with no unstirred layer as a function of time. Solid lines show potassium concentration with time. Dotted lines indicate hypothetical changes in light chain phosphorylation. Phosphorylation ordinates are adjusted so that 60 mM K^+ results in 0.65 mol P_i/mol light chain and resting values of phosphorylation are 0.10.

timate of the contribution of diffusional delays to activation rates in tissues. However, a limited analysis can be made with four assumptions. 1) Crossbridge activation measured by the rise in myosin phosphorylation subsequent to K^+-depolarization is a rapid event compared to the rate of K^+ diffusion into the strip. 2) Cells in the carotid media are not electrically coupled. 3) The concentration of adrenergic nerves at the adventitial-medial junction allows an assumption that neural excitation will not precede excitation by K^+-depolarization. 4) We also assume a situation in which myosin phosphorylation rises concomitantly with depolarization to a maximal activation achieved at 60 mM K^+ at the cell membrane and then declines. The cellular rate of decline is unknown and the time courses were chosen (fig. 4) to approximate the rates at which shortening velocities fall with time in electrically stimulated (homogeneously activated) tissues[11]. This approach is based on the tight correlation between V_0 and phosphorylation found under steady-state or quasi-steady-state conditions in this tissue[4].

The time course of myosin phosphorylation in the entire tissue stimulated by an agonist added to the muscle chamber was calculated (with the above assumptions) using a reiterative program which determines the level of phosphorylation at each position across the strip at a given time from the concentration profile and phosphorylation time course for the agonist. Values of phosphorylation are summed across the strip at each time. This results in a phosphorylation transient, the time course of which will be a function of agonist diffusion times (fig. 5).

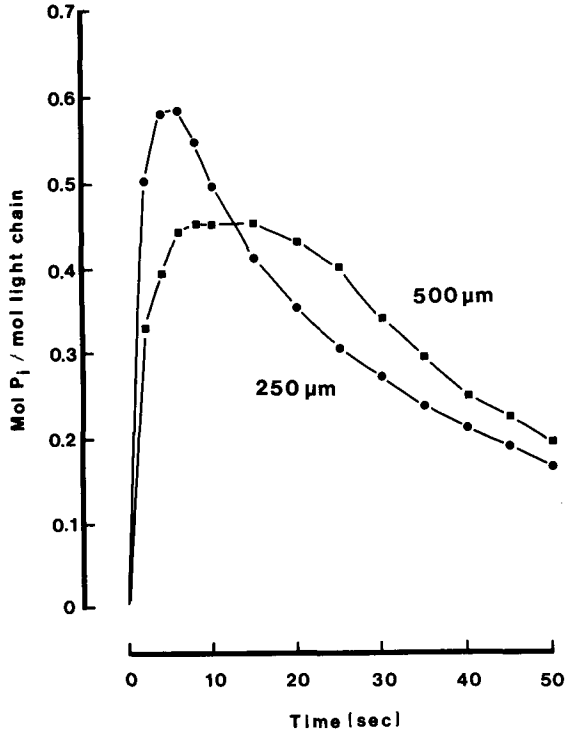

Figure 5. Calculated time courses of light chain phosphorylation in tissues at different times after stimulation. Curves are derived by summing values of phosphorylation across the strips shown in figure[4]. This analysis neglects the diffusion boundary layer effects which would further slow the transients (compare with experimental data in fig. 2).

Discussion

Agonist diffusion times contribute to activation rates in smooth muscle tissues which are not electrically coupled[5,28]. However, the very slow rates of stress development exhibited by large arterial tissues implied that diffusion times are rapid compared to excitation-contraction coupling events or crossbridge cycling rates. The recent observations of Ca^{2+} transients in agonist-stimulated arterial tissues [29,30] and evidence that these are associated with transients in myosin phosphorylation[3] and in cross-bridge cycling rates [4,10] show that excitation-contraction coupling may be rapid. Thus, the cellular myosin phosphorylation transient and the development of the latch state characterized by very low shortening velocities may be fast relative to agonist diffusion. Considerable evidence indicates that estimates of activtion which are tissue averages, such as myosin phosphorylation, stiffness, or active stress, provide distorted kinetic characterizations[3]. The experiments described in this study were undertaken to estimate the magnitude of the problems introduced by agonist diffusion and heterogeneous activation.

Diffusion and activation rates

Both dS/dt and the time course of the velocity transient were slowed in tissues thicker than 400 µm (figs 1 and 2). This indicates the presence of significant diffusion limitations. Similar observations on the dependence of rate of stress development on tissue size were made by Kato et al.[27] for Ca^{2+}-stimulated, K^+-depolarized uterine muscle sheets. Such a delay was also observed in comparing rates of phosphorylation measured in thick[4] and thin (fig. 3) tissues when light chain phosphorylation peaked at 30 and 16 sec, respectively.

In thin strips the time course of the velocity transient would appear to be independent of the diffusion path since over a 200 µm range of thicknesses the TPV was constant (fig. 2). However, this conclusion is inconsistent with other observations on thin tissues. 1) In thin tissues, activation as assessed by shortening velocity occurs more rapidly than myosin phosphorylation. 2) A theoretical analysis of the potassium concentration profiles across a thin strip (fig. 4) suggests that the time required for K^+ to reach maximal stimulating concentrations at the center is comparable to the TPV, and is probably longer with a realistic assumption for the diffusion boundary layer thickness.

The early onset of the shortening response indicates that complex events involved in agonist action on the membrane, Ca^{2+} increases in the myoplasm, Ca^{2+} binding to calmodulin, activation of kinase and myosin phosphorylation are rapid processes in the carotid smooth muscle cells. Shortening velocities will not reflect a simple tissue average to the extent that the rates of activation of more superficial cells are measured. A.V. Hill[23] has analyzed the situation when rates of shortening (or levels of activation) differ within the preparation, and the resultant effect on the force-velocity relation. When the muscle is shortening with velocity V, the cells (central unstimulated cells in the case of the carotid media) with a velocity $< V$ become slack and exert no force, while cells with a velocity $> V$ are taut and contribute to force developed. At early times of stimulation, an afterload of a fixed fraction

of S_o will impose a disproportionately large load on a few activated cells and velocity will be low. At some time a sufficient number of superficial cells will be maximally activated and able to shorten rapidly against the afterload, while unstimulated central cells go slack.

Shortening velocity measurements on tissues at a low constant afterload have the potential to reveal the early cellular responses, where values of force or phosphorylation are a damped average of cellular responses across the tissue. Better time resolution should be obtained by quick-releases to loads less than the 0.1 S_o used in this study or by estimates of V_o using the force-velocity relation. Estimates of shortening rates, however, remain a fairly qualitative assessment of activation, since the relative load on individual cells falls rapidly with time as more cells are activated. The onset of shortening will also be limited by diffusion times across a boundary layer whose thickness is probably independent of tissue dimensions. It is possible that receptor activation, Ca^{2+} release and myosin phosphorylation are rapid events compared to diffusion through the boundary layer[15,26]. This would preclude precise resolution of the kinetics of cellular activation by mechanical measurements of agonist-activated arterial tissues.

Other delays involved in the development of force by arterial tissues can be estimated. The use of a simplified model of contraction in the form of a calculated myogram can give a rough estimate for delays seen in tension development due to stretching of the series elastic element by the contractile element. The rate of rise of externally measured force can be estimated using a two-component mechanical analog model of an undamped passive series elastic element in series with an active contractile element[22]. Using measured parameters of the force-velocity relation and series elastic compliance of hog carotid artery[20], the calculated tension time course has a time to half-maximal stress ($t_{0.5\ S_o}$) of 1.1 sec and $t_{0.99\ S_o} = 4.0$ sec. This is fast compared to measured values of 15 and 135 sec, respectively (fig. 1). The difference may be attributed to two factors. 1) The calculated tension time course of a cell assumes instantaneous activation. However, agonist diffusion delays will slow activation of interior cells in tissues. 2) The phosphorylation transient and subsequent development of the latch state associated with very slow rates of crossbridge cycling may delay the attainment of steady-state S_o.

The present analysis suggests that the rate of force development in the carotid media cannot be limited by the steps involved in E–C coupling or the time required to stretch the series elastic component by the phosphorylated contractile system. Delays due to agonist diffusion can be minimized but never eliminated in studies of the tissue response to agonists. These delays will produce significant effects on the rates and magnitudes of the phosphorylation transients in tissues employed in in vitro studies.

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Current problems in smooth muscle mechanics

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Key words. Smooth muscle; mechanics; length-tension relation; force-velocity relation; mechanical transients; cross-bridge properties.

The primary aim of studies on muscle mechanics is to establish relations between variables such as length, force, stiffness, velocity, and time in the relaxed state and during contraction. It is often useful to describe these relations by fitting them to mathematical equations or in terms of analogue models, but the ultimate goal must be to explain the relations on the basis of the biochemical and ultrastructural properties of the tissue.

Among the mechanical relationships that have been studied in muscle research the *length-tension relation* is the most well known as it received attention already in the end of the 19th century. However, a proper understanding of its characteristic features was not obtained until the launching of the sliding filament mechanism of contraction in the mid 1950ies, and accurate quantitive comparisons between active force and filament overlap in the sarcomer were first reported in 1966[8].

Detailed determination of the length-active tension curve in smooth muscle is particulary difficult due to the fact that there is an appreciable passive force already at the length (L_0) where active force is maximal and that passive force rises steeply above this length. This is exemplified by figure 1 taken from the work of Herlihy and Murphy[11] on pig carotid smooth muscle. Note that the spread of data increased very much above L_0 and that the descending curve could not be followed down to zero force. Mulvany and Warshaw[20] studied length-tension relations of arterial smooth muscle from small vessels and used a quick release technique to separate passive and active forces above L_0. They found that the descending part of the active curve was reasonably straight and extrapolated to zero force at about 1.8 L_0.

It is considered that the early compliant part of the passive length-tension curve in blood vessels reflects the stretching of elastin fibers and that the later, stiffer part is due to collagen[21]. If it is true that the smooth muscle cells in spite of their cytoskeleton of intermediate filaments do

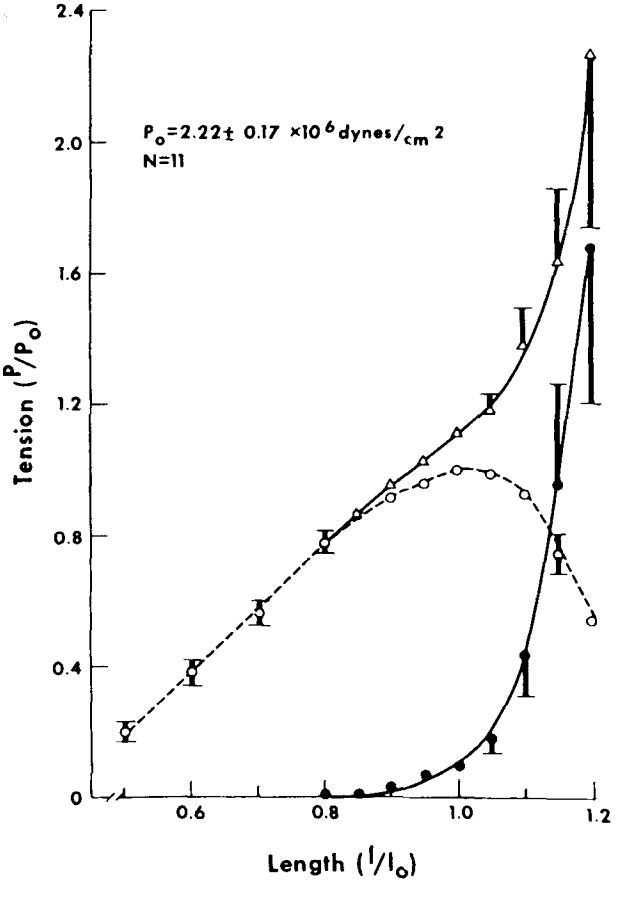

Figure 1. Length-tension relations of carotid artery smooth muscle. The top curve (triangles) shows tension as a function of length for the potassium-depolarized muscle in physiological salt solution containing 5 mM $CaCl_2$. The bottom curve (solid circles, solid line) is the resting tension curve. The difference between the two curves is the active length-tension curve (open circles, broken line) (from Herlihy and Murphy[11]).