Static and Dynamic Components in the Vascular Myogenic Response to Passive Changes in Length as Revealed by Electrical and Mechanical Recordings from the Rat Portal Vein

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ABSTRACT

The effects of static and dynamic passive stretch and shortening on electrical activity and active force were analyzed in the isolated rat portal vein. Static stretch by 40% of muscle length evoked moderate excitatory effects with enhanced mechanical activity and an average increase in spike discharge of 12% above the control value of 55 ± 2.6 spikes/min. The dynamic responses studied at various rates of length change (dL/dt) over the range between -12 and +12 mm/min, i.e., -3 and +3% muscle length/sec, were much more pronounced. Active force and spike activity showed graded increases with increasing rates of stretch. The electrical activity reached a value of 180 spikes/min (≈ 325% of control) at 5 mm/min; this frequency was then maintained for stretch rates up to 12 mm/min. Mechanical activity during stretch was further reinforced by the shift along the length-tension diagram. Passive shortening at rates from -1 to -12 mm/min caused graded decreases in mechanical and electrical activity below the control levels, complete inhibition being observed at the latter dL/dt. Blockade of α and β receptors indicated that the responses were myogenic in nature. The findings seem to provide direct support for the myogenic hypothesis of vascular tone and responses to stretch of the vascular wall, but they indicate that emphasis should be placed on the dynamic characteristics of the stimulus rather than its static nature. This emphasis constitutes a new concept in the myogenic control of the peripheral circulation.

KEY WORDS vascular smooth muscle in vitro isometric force passive lengthening and shortening sucrose-gap technique spike discharge myogenic hypothesis autoregulation

Enhancement of contractile activity in the smooth muscle of blood vessels through a direct stimulating action of the distending intravascular pressure was proposed by Bayliss (1) in 1902 as a possible mechanism contributing to vascular tone and reactivity. Experimental support for this mechanism has been obtained from hemodynamic and vital microscopy studies (2-5) so that it is now widely appreciated as an important factor in the development of basal vascular tone and in active responses to changes in blood pressure, e.g., in autoregulation of blood flow. The cellular events behind these myogenic reactions have been tentatively discussed in light of studies on smooth muscle which have revealed increased frequency of action potentials and increased contractile activity in response to passive stretch. This information has been derived mainly from experiments on nonvascular smooth muscle (6-8), but there are also a few studies on isolated blood vessels which have indicated an active contractile response to stretch (9-12). In two of these studies (11, 12), indications of increased frequency of propagated electrical activity were found during the stretch response.

Synthesis of observations from in vitro and in vivo experiments led to the formulation of the myogenic hypothesis by Folkow (13). In brief, the myogenically active vascular smooth muscle is considered to act as a mechanoreceptor in which distention, via effects on a pacemaker mechanism, causes facilitation of impulse discharge propagated to the neighboring muscle effector cells. The net effect of such a mechanoelectrical coupling is changes in the frequency of spike generation in response to deformation and attendant active changes in vascular tone.

The myogenic vascular reactions in vitro and in vivo have mainly been considered in relation to static stretch, which is repre-
VAScular Response to Dynamic Stretch

sent in vivo by a shift in blood pressure from one steady level to another. A recent investigation (14) has suggested, however, that the basal tone and the myogenic responsiveness of peripheral resistance vessels are subjected to additional stimuli through the dynamic component in the repetitive distention caused by arterial pulse pressure. In isolated vascular smooth muscle there has also been some indication of a rate-sensitive mechanical response following rapid lengthening (9, 10). The cellular mechanism behind such dynamic responses to stretch is unknown.

The aim of the present study was to investigate in quantitative terms the electrical and mechanical responses of vascular smooth muscle to passive stretch with special emphasis on the importance of the rates of induced changes in length, both positive and negative. The isolated rat portal vein was used as a model. We found that the electrical and contractile activity of the vessel depended on muscle length and were strongly influenced by variations in the rate of change in length \(\frac{dL}{dt}\) in a well-defined, graded manner.

Methods

Rats of the Sprague-Dawley strain with body weights of 350–400 g were used. After the rat had been killed by cervical fracture, a section of the portal mesenteric vein about 1.5 cm long was dissected out. Adventitial connective tissue surrounding the portal vein was gently removed under a dissection microscope to reduce the amount of passive elastic elements in parallel with the muscle cells. The vein was mounted in a sucrose-gap apparatus for simultaneous recording of electrical and mechanical activity in the longitudinal smooth muscle as described previously (15). The hepatic end of the preparation was mounted vertically and connected to a Grass FT03 force-displacement transducer which could be raised or lowered by a micrometer screw, thus permitting passive changes in muscle length. It was essential for the present experiments that the lower end of this active part of the preparation was well fixed at the orifice of the sucrose-gap tube so that stretch could be applied without appreciable slipping or mechanical disturbance of the electrical recording. Owing to the relatively greater thickness of the hepatic part of the vein, it was possible in most cases to obtain satisfactory fixation by gently pulling the preparation into the sucrose-gap tube. Preparations in which this procedure failed were discarded.

Even with good fixation, it is difficult to exclude entirely mechanical influences on the d-c potential across the sucrose-gap; therefore, no attempts were made in this study to evaluate changes in the level of resting membrane potential. Attention was focused, instead, on the phasic potential changes. For this purpose, the differential amplifier which received the signals from the sucrose-gap electrodes was coupled in an a-c mode. Furthermore, the output was filtered so that, in the recordings presented in this paper, only electrical events corresponding to sine wave frequencies above 5 Hz were fully displayed. The upper limit was set by the frequency response of the recorder, which attenuated to half-amplitude at 35 Hz. Between 0 and 5 Hz there was an approximately proportionate decrease in amplitude with decreasing frequency. The signals from the force transducer and the sucrose-gap electrodes were recorded on a direct-writing oscillograph (Grass polygraph) and stored on magnetic tape (Tandberg Instrumentation Recorder, 100) for later replay.

Quantitative assessment of the active force developed by the smooth muscle was made using an electronic integrator. This unit measured the area under the force curve above the control resting level. Data on contractile activity during passive changes in muscle length were obtained by subtracting the area corresponding to the increase in passive force from the total integrated value. The increase in passive force during lengthening was readily obtained at low rates of stretch from the mechanical recording, since clear-cut intervals of rest still occurred between the phasic contractions (Fig. 1, bottom left). Corresponding curves compressed in time gave the area of passive force for higher rates of stretch. Active force is given as a time average (obtained as dynes \(\times\) min/min).

The electrical activity, which in the portal vein is characterized by variable bursts of action potentials, was analyzed in quantitative terms by an electronic spike counter. This device was triggered by the sucrose-gap signal each time the latter increased beyond a preset level above the base line. The trigger level was adjusted by checking the counter against the direct recordings of electrical activity obtained at high paper speed (Fig. 1, bottom left) so that biological potential changes were counted without disturbance from base-line noise. Limitation of the counting range to intervals > 30 msec prevented undue repetitive triggering by high-frequency noise in the electrical recording. The output from the counter was recorded on the Grass polygraph. Resetting occurred automatically after 100 impulses had been sampled, or it could be done manually. The spike frequencies obtained in this way from the sucrose-gap recordings are not necessarily representative of the spike activity in each individual muscle cell, since the extracellular technique might register potentials from several muscle bundles.

After the preparation was mounted in the sucrose-gap apparatus, it was allowed to accommodate for at least 1 hour before the actual experiment started. During this period, the active hepatic part of the preparation was maintained at a low preload (= 70 dynes); the length of this segment was then between 6.0 and 7.0 mm in the
different experiments. The vessel was continuously superfused with standard Krebs solution of the following millimolar composition: NaCl 122, KCl 4.7, CaCl₂ 2.5, MgCl₂ 1.2, NaHCO₃ 15.5, KH₂PO₄ 1.2, and glucose 11.5. This solution was bubbled with a 96% O₂-4% CO₂ gas mixture giving a pH of 7.3, and temperature was kept at 37°C. By the end of the accommodation period, the muscle had assumed the regular pattern of electrical and mechanical activity typical for the rat portal vein.

In the Results, spread of data is expressed as the standard error of the mean.

**Results**

After a few pilot experiments performed to assess the experimental approach, observations were made on five different portal vein preparations. The experimental procedure usually adopted in this series of experiments is exemplified in Figure 1, which shows the effects evoked by passive stretch and shortening of the portal vein between 6.0 and 8.5 mm (= +40%) at two different rates, 1 mm/min (top) and 5 mm/min (bottom). The original tracings illustrate the mechanical and the electrical activity of the smooth muscle preparation; the latter event is depicted as both bursts of spikes (a-c recording) and number of spikes during each experimental step (spike-counter recording). Below these tracings, the derived values for active force (time average) and spike activity per unit time are given.

In the control period before stretch (preload about 70 dynes), the portal vein showed its typical spontaneous electrical spike discharge in bursts and phasic mechanical responses, in this case corresponding to 41 spikes/min and an active force of 14 dynes (Fig. 1, top). The subsequent passive stretch of the muscle preparation at a constant rate of 1 mm/min (2.5 mm/150 sec) evoked clear-

![Figure 1](http://circres.ahajournals.org/)

**Effects of dynamic and static passive stretch and shortening on mechanical and extracellularly recorded electrical activity in the isolated rat portal vein.** Lengthening from 6.0 to 8.5 mm at a rate of 1 mm/min (top) and 5 mm/min (bottom) evoked marked excitatory effects, most pronounced for the higher dL/dt, which also caused an afterdischarge (see calculated data for active force and spikes/unit time). At a constant increased length, activity stabilized at levels somewhat above the control values at initial length. Phasic shortening at rates of −1 and −5 mm/min caused inhibitory effects, in the latter case complete abolition of activity. The left part of the bottom section shows recordings taken at high paper speed of a single contraction, burst of spikes, and spike-counter output.
cut increases in both electrical discharge (94 spikes/min) and active force (78 dynes). After the muscle had reached the final length of 8.5 mm, activity tended to decline and become more regular, but it was still enhanced in comparison with the control period as evidenced by the somewhat longer duration of the electrical bursts and the larger contractions. Shortening of the muscle at the rate of 1 mm/min caused some inhibition of the activity, as can be seen from the less frequent contractions and the decline of spike discharge. On return to the original muscle length, control activity was gradually resumed.

Stretch at the higher rate of 5 mm/min (2.5 mm/30 sec) (Fig. 1, bottom) caused a pronounced increase in mechanical and electrical activity with the active force increasing from 11 to 113 dynes and the spike discharge from 42 to 166 spikes/min. In this case there was also a transient electrical afterdischarge, for which quantitative data are given separately. In the subsequent steady-state period of static stretch, mechanical and electrical activity stabilized at lower levels. Passive shortening of the portal vein at the rate of ~5 mm/min caused complete inhibition of the mechanical and electrical events, but these events reappeared when the muscle had reached its control length. These results show that there is a static as well as a dynamic component in the response of the portal vein to passive stretch.

The described responses to change in length were not altered by the addition of $10^{-6}$M phenoxybenzamine and $10^{-6}$M propranolol to the medium, concentrations which effectively block the $\alpha$ and $\beta$ receptors. Furthermore, the same pattern of contractile response to dynamic stretch was observed in experiments in which electrical activity was not recorded, showing that the excitatory effects were not artifacts evoked by mechanical derangement of the mounting in the sucrose-gap tube.

The quantitative relation between the rate of stretch and the magnitudes of the mechanical and electrical responses of the vascular smooth muscle is elucidated in greater detail in Figure 2, which shows the dynamic responses to passive stretch (40%) at six different rates between 1 and 6 mm/min. There was a graded increase in the mechanical and electrical activity with increasing rates of stretch; at the highest rates, the muscle was almost continuously active. Integrated active force and spike activity per unit time changed in parallel during the various periods of dynamic stretch; the ratio of active force to spike activity was quite constant. The ratio in this particular preparation averaged $0.64 \pm 0.01$ dynes/spike. In the control situation, this ratio was only about $0.30$ dynes/spike due to the muscle's active length-tension characteristics (see Discussion).

![Figure 2](http://circres.ahajournals.org/). Effects of passive lengthening (40%) of the isolated rat portal vein performed at graded rates of stretch. The electrical and mechanical excitatory responses increased in a graded fashion with increasing rates of stretch.

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Compiled data (means ± SE) for the electrical response of the isolated rat portal vein to passive dynamic changes in length (40% elongation and subsequent shortening) at rates ranging from -12 to +12 mm/min. Note the marked increases in spike discharge during phasic stretch and the graded decreases below the control level (dL/dt = 0, initial length) during phasic shortening. Data were obtained from five muscle preparations; the number of observations for each point is given below the abscissa.

Dynamic response to passive stretch (1-12 mm/min) in terms of changes in spike activity per unit time. This figure also includes the effects evoked by passive muscle shortening at rates between -1 and -12 mm/min. The data refer to observations from five portal vein preparations stretched by 40 ± 1.4% above their control lengths prevailing at a preload of about 70 dynes and subsequently shortened by the same amount. The number of observations for each dL/dt value is given at the bottom of the figure.

In the control situation at initial length (dL/dt = 0), the spike discharge of the portal vein averaged 55 ± 2.6 spikes/min. Spike activity increased greatly and in a graded fashion with increasing rates of stretch to a value of about 180 spikes/min at a dL/dt of 5 mm/min, after which the curve leveled off for stretch rates up to 12 mm/min. Conversely, there was a graded decrease in spike activity below the control level when the muscle preparation was exposed to passive shortening at rates between -1 and -12 mm/min. At the latter dL/dt value, spike discharge was zero, but such complete abolition of the electrical (and mechanical) events was occasionally seen at lower rates of shortening (Fig. 1, bottom).

The data in Figure 3 refer to the spike activity sampled solely during the period of phasic change in muscle length, but, as exemplified in Figure 1 (bottom) there was often an afterdischarge present immediately on completion of the stretch, especially if the preceding change of muscle length was fast. The duration of such an afterdischarge tended to increase gradually with increasing rates of stretch up to 4-5 mm/min and averaged 15 seconds at these and higher dL/dt values. The spike discharge rate within the afterdischarge period seemed, however, to be independent of the rate of stretch and averaged 188 ± 11 spikes/min. Even though this afterdischarge occurred after completion of the stretch procedure, the pattern of activity (Fig. 1, bottom) strongly suggests that the phenomenon is a component of the dynamic stretch response.

The spike activity recorded in the steady-state period of static stretch (40% increase in muscle length, passive force of 370 dynes) averaged for 41 observations 64 ± 2.5 spikes/min. This value is significantly higher than that (55 ± 2.6 spikes/min) observed in the control period at initial length (P < 0.01), showing that the portal vein indeed is sensitive to static elongation, even if this component of the stretch response is much less impressive than the dynamic component.

Discussion

The present study demonstrated an increase in the electrical and mechanical activity of the isolated rat portal vein in response to passive stretch and inhibitory effects in response to passive shortening. Moderate excitatory effects were found during static stretch, i.e., in the steady-state situation of constant increased length, but the changes in activity were more pronounced in the dynamic phases of passive lengthening or shortening. Apparently, these responses can be ascribed to deformation of a sensing structure, but it is not possible to decide from the present study whether the primary stimulus for this element is represented by the change in length (dL/dt) or in passive force (dP/dt). The described reactions were not affected by blockade of either α or β receptors, indicating that they were not related to liberation of endogenous norepinephrine. It appears therefore that the effects observed are myogenic in nature; hence, they will be discussed in light of present knowledge of the intrinsic organization of the effector.
The excitatory process in the smooth muscle of the portal vein is complex in that at least two electrophysiological periodicities can be distinguished: one is represented by the duration of the bursts and the intervals between them and the other by the spike activity within an individual burst. Observations of a gradual decline in membrane potential during the quiescent periods between the bursts (15) indicate that the frequency and the duration of the phasic contractions are determined by a pacemaker mechanism. With regard to this rhythm, the portal vein preparation therefore behaves essentially as a single-unit smooth muscle, although the size of the unit (the number of cells participating in the mechanical response) can differ somewhat between the individual contractions. Also the temporal pattern of spike activity varies between the individual bursts and between different parts of the preparation. As mentioned, the spike frequencies recorded in the present study do not necessarily represent the electrical events in each individual cell. When large preparations of portal mesenteric veins (about 20 mm long) are studied, the individual spike cannot be followed over the entire strip and activity in such cases seems to be regulated by multiple pacemakers (16).

The present experiments indicated that the pacemaker mechanism responsible for burst activity was influenced to a large extent by passive stretch so that frequency and duration of bursts were both increased (Figs. 1 and 2). Spike frequency within the bursts appeared to be less affected. The mechanical activity of the portal vein was obviously increased by passive stretch in relation to the enhanced electrical activity, but it was further reinforced by the shift along the length-tension curve of the contractile system. The latter effect was quite pronounced in the present experiments (Fig. 2), which were carried out with a low preload in the control situation; in this low range of passive force, the ability of the muscle to produce active force is known to increase markedly with length (17).

Some possible implications of the present findings for the regulation of vascular tone in vivo should be considered. Spike activity may not be a universal feature of small blood vessels (18), but single-unit behavior appears to be a dominant characteristic of the smooth muscle in arteriolar resistance vessels and precapillary sphincters (5, 19, 20). The portal vein, therefore, may serve as an acceptable model for the study of myogenic vascular responses in general, even though the size of the single unit, i.e., the population of cells participating in synchronized responses, may be unusually large in this particular vessel. The present findings, then, may be taken as direct support for the myogenic hypothesis of vascular tone and vascular responses to stretch. In current discussions, the myogenic hypothesis has mainly been concerned with the influences of static stretch, but the present study indicates that emphasis should be placed on the dynamic features of the stimulus. The question then arises whether this dynamic myogenic stretch response can have a corollary in the in vivo situation.

In the present study, the smooth muscle preparation was stretched by a total of 40% of its initial length at rates of 1–12 mm/min, i.e., by about 0.25–3.2% muscle length/sec. The dynamic excitatory response was evoked very shortly after the beginning of stretch and was then present throughout the period of lengthening (Figs. 1 and 2). It was, in fact, clearly discernible at length changes of less than 5% and often triggered at the very commencement of stretch. Vascular distensibility in vivo has been determined for an idealized "unit resistance vessel" in skeletal muscle (21). This study indicated that, within the normal range of transmural pressure, the radial distention of a resistance vessel with normal tone is about 0.33%/mm Hg mean distending pressure. Arteriolar distensibility in the omentum, as determined by vital microscopic techniques, is on the same order of magnitude (22). Such data imply that an increase in mean distending pressure of 30 mm Hg would cause a passive distention of the resistance vessels of about 10%. If such an event occurred over a period of 20 seconds, the rate of radial vascular distention would be 0.5%/sec; if it occurred in 5 seconds, the stretch rate would be 2%/sec. These values correspond to the present in vitro stretch experiments at rates of 2 and 8 mm/min. Such considerations indicate that the observed dynamic component of the myogenic response to changes in length can be a major factor responsible for the adaptive alterations of myogenic tone that occur in...
resistance vessels and precapillary sphincters when transmural pressure gradually increases or decreases (3, 5, 13).

A recent in vivo observation of enhanced vascular tone and myogenic reactivity in response to pulse pressure distention (14) might also be explained in terms of a dynamic response to stretch. Arteriolar radial distention in response to the local pulse pressure oscillation is on the order of 1% (22). This value implies length changes at rates of about 3%/sec for a systolic period of 0.3 seconds and -1.5%/sec for a diastolic period of 0.7 seconds. Such events would correspond to the present stretch experiments at rates of 12 mm/min and -6 mm/min, respectively. The net effect over the entire cardiac cycle of such pulse pressure–induced length oscillations might very well be an increase in vascular tone considering that the spike discharge curve (Fig. 3) is much steeper for positive values of dL/dt than it is for negative values and that an afterdischarge is present at high rates of stretch. The relative sluggishness of the smooth muscle contractile system compared with the spike generation would have a damping influence and prevent undue rapid undulation of vascular tone during the cardiac cycle.

The effects described in this paper should be clearly distinguished from those evoked by very rapid changes of length produced by quick-stretch techniques or high-frequency vibrations (23, 24) which can cause inhibition of vascular smooth muscle, apparently via direct interference with the contractile machinery (24).

Myogenic reactivity (e.g., myogenic autoregulation of blood flow) has been considered mainly in relation to changes in blood pressure from one steady level to another. No doubt, such a static component of the myogenic response does exist, as also evidenced by the present study, but the dynamic component apparently is much more prominent. This concept can explain how prompt myogenic vasoconstrictions or vasodilations are accomplished during the phasic period of a blood pressure change and how such responses are graded in relation to the rate of the pressure change. Such precise effects are implicit in the phenomenon of blood flow autoregulation. If the static myogenic constrictor response evoked during the plateau of a blood pressure rise failed to maintain vascular tone at the new increased level, the vessel would again be transiently distended which, in turn, would create the stimulus for a repetitive dynamic myogenic constrictor response. The damping and asynchrony of such cyclic variations in tone in different sections of the vascular tree are factors that contribute to the maintenance of an overall relatively stable level of blood flow in the tissue. It has sometimes been said that a positive feedback mechanism is inherent in the myogenic theory (the pressure rise causes constriction and an increase in resistance which, in turn, causes an additional pressure rise, etc.). The present data show that any extreme effects of such a mechanism will not ensue, since there is an upper biological limit of the dynamic response at high rates of stretch (Fig. 3). Furthermore, the chemical metabolic control system acts as a brake on the myogenic control, since intense vasoconstriction is counteracted by the consequent local accumulation of vasodilator metabolites (25).

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