High shortening velocity of isolated single arterial muscle cells[1]

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Summary. Surprisingly high shortening velocities (< 200 msec contraction-relaxation cycles) were found in isolated vascular muscle cells cultured from rat or chick aorta. All of the small fraction of cells with such quick contractions had membrane excitation by short duration spikes, rather than the slower graded depolarization of the other cells which produced 20-fold slower contractions.

It is generally believed that contraction of mammalian nonstriated muscle including that found in blood vessels must be slow compared to striated muscles, such as skeletal muscles of the twitch type. One proposed explanation is that the factor limiting the speed of contraction is the contractile protein interaction in nonstriated (smooth) muscles because the contractile protein activation step is a direct myosin activation rather than the troponin activation system found in other vertebrate muscle contractile proteins[2]. However, the work of Bagby has shown that the velocity of contraction is higher in isolated single cells than in a smooth muscle strip[3]. In such single cells, the contractile filament reorientation demonstrated by birefringence photomicrographs showed that the contractions sweep across the cell, suggesting that quickness of the activation step rather than the calcium protein interaction may be the rate limiting factor. Furthermore, Mulvany and Halpern[4] have recently shown that contraction of the nonstriated muscle of small arteries can be more rapid than previously expected, with a time to half-peak tension of 210 msec. In the present experiments, I found that isolated single arterial muscle cells contracted with even greater shortening velocities when membrane spiking appeared.

Methods. The thoracic aorta of embryonic or neonatal chick or rat was the source of arterial muscle cells for these experiments. The cells were dispersed by sequential treatment with collagenase and trypsin and cultured in medium based on M199 and containing 15% horse serum[5]. The isolated arterial muscle cells then spontaneously attach to collagen or polylysine-coated glass coverslips, immobilizing themselves to allow experimentation, but showing clearly observable movement of the contractile apparatus[6,7]. Membrane electrical events initiating the contractions were measured by intracellular microelectrodes which allowed simultaneous filming of cell contractions[8].

Results and discussion. The isolated arterial muscle cells showed spontaneous contractions of one of 2 types. The majority of the cultures contained only cells contracting slowly with a cycle duration of 2–30 sec, triggered by slow and graded depolarization (S mechanism), as is found in isolated strips of the aorta. The minority of the cultures (8 of 120) contained only cells contracting more rapidly than expected for vascular muscle. The quick contractions consisted of a twitch-like shortening that was often less than 200 msec in duration and closely followed membrane electrical spikes of approximately the same duration (F mechanism). For all cultures, contractions began within 24–96 h. F mechanism contractions appeared within 24 h in 2 cultures. The differences between the generally known slow (S) activation mechanism and the newly found fast (F) activation mechanism are summarized in the table. The most striking difference on microscopic observation was the difference in contractions (table, characteristics 1–3), especially the contraction duration, which showed a 20-fold difference.

Detailed analysis of an F mechanism contraction is shown in figure 1. A single cell is shown at high magnification in sequential frames from a cine film. The shortening of the contractile apparatus is measured from movement of its cytoskeletal attachment points, which are emphasized by black dots in figure 1. The single cell F contractions consisted of contractile apparatus shortening of up to 10%. Time motion analysis of enlarged projected images analyzed frame-by-frame showed that the modal contraction duration was 200 msec with a minimum of 167 msec and a maximum of 290 msec. Figure 2 shows an expanded single F contraction and underlying electrical spike. The spontaneous contraction frequency was 10–15/min during maximum activity. Periods of inactivity lasting up to 15 min were interspersed between active periods. During such cyclical variations in spontaneous frequency, the contraction cycle duration did not detectably increase, but maintained its mean of 200 msec. In contrast to the rapid (F) mechanism contractions, the slower (S) mechanism contractions were of longer duration and greater magnitude; and the spontaneous contraction frequency was lower even during most active periods (characteristics 1–3 of the table). The individual S contractions lasted about 20 times longer than the F contractions. The S contraction duration and amplitude increased to a maximum of 15 sec and 40% as spontaneous frequency decreased.

The different membrane activation mechanisms are contrasted in characteristics 4–6 of the table. The F contractions were activated by electrical spikes arising from spontaneous depolarizations. The electrical spike associated with the F contraction in figure 2 shows that the spike duration was nearly equal to the contractile duration. Spike threshold was consistently about −30 mV and maximum membrane potential (E_m) was about −50 mV. The spikes always occurred singly and without any evidence of a plateau, instead approximating nearly perfect double exponentials. In all cells observed contracting quickly, a spike activation mechanism was found. In contrast, S cells were always activated by graded depolarization that occurred more slowly. The depolarization time course paralleled the S contraction cycle. S contraction occurred as a continuous

2 different arterial muscle cell contraction mechanisms

Characteristic	S mechanism	F mechanism
1. Contraction duration, sec		
Mean	4	0.20
Minimum	1.5	0.17
Maximum	15	0.29
2. Contractile apparatus shortening	20–40% Lr	5–10% Lr
3. Peak spontaneous contraction frequency, min^{-1}		
Mean	2	10
Minimum	0.1	4
Maximum	4	15
4. Membrane activation, type	Graded	Spiking
duration, sec		
Mean	4	0.20
Minimum	1.5	0.17
Maximum	15	0.29
5. Threshold E_m, mV mean ± SEM (number)	None (32)	−30 ± 0.5 (6)
6. Resting E_m, mV mean ± SEM (number)	−45 ± 2 (15)	−40 ± 2 (6)

function of E_m between −40 and −20 mV. The average resting E_m was more negative than that of the F cells. These experiments showed that a fundamental change in membrane excitation mechanism can occur in vascular muscle when the cells are placed in primary culture. Cells that converted to spike excitation associated with quick shortening were dramatically different than cells showing normal graded excitation associated with slow shortening. The fact that such high shortening velocities can occur reveals additional contractile capabilities of arterial vascular muscle cells normally hidden by a slow membrane excitation mechanism.

The graded depolarization mechanism in vascular muscle has been demonstrated in a large variety of blood vessels, and is characteristic for the intact aorta[9]. However, these aortic muscle cells took on a spike-generating capability. This conversion is similar to that which was found in a gradedly responsive slow skeletal muscle[10] and in *Limulus* myocardium[11]. Induction of spiking by norepinephrine and elevated Ca^{++} was also reported in young rat aorta[12], although high shortening velocity contraction would not have been detected in isolated muscle strips. Since the spikes that occurred in these cultured aortic cells are similar in shape, although 4 times quicker than those seen in normally spontaneously active vascular muscle[13], there may be a fundamental but normally latent all-or-none membrane excitation mechanism, even in gradedly activated vascular muscle.

The quick contractions that were observed in the spiking cells are hypothesized to be caused by the altered membrane excitation mechanism for 3 reasons. Quickly contracting cells: 1. were not found in the same culture dish with slowly contracting cells, as might be expected if the former represented preformed pacemaker cells; 2. appeared at the same time as spike excitation; and 3. followed membrane potential time course exactly enough to be caused by it. The close correlation of membrane and contractile events could be made here because cultured cell contractile apparatus movements can be individually observed during electrical recording. The contractile apparatus event was therefore not obscured by elasticity or the delays in cell-to-cell activation[14], which are inherent in an isolated or intact blood vessel.

Morphologically, the F and S types of vascular muscle cells were identical, as is shown in figure 3. The F cells could be found either as single cells or small aggregates. Cultures of cells with no distinguishable differences in size, shape, length, or observable features showed only the S contractions. Single cells only occurred at 1–4 days. The large cell sheets which represent cell reassociation (not shown here) were always found to be the S contraction type. Although contractile cells could be recognized during the 1st day by their phase contrast halo and greater thickness measured by differential interference contrast focal plane difference, after 1–2 days the contractile cells lost these features and could only be identified by their ability to contract.

These cultures of vascular muscle, which have been optimized to maintain full physiological characteristics of the muscle cells[18] have quite different properties than the subcultures of vascular muscle, which have been shown to convert to a noncontractile state usually associated with cell

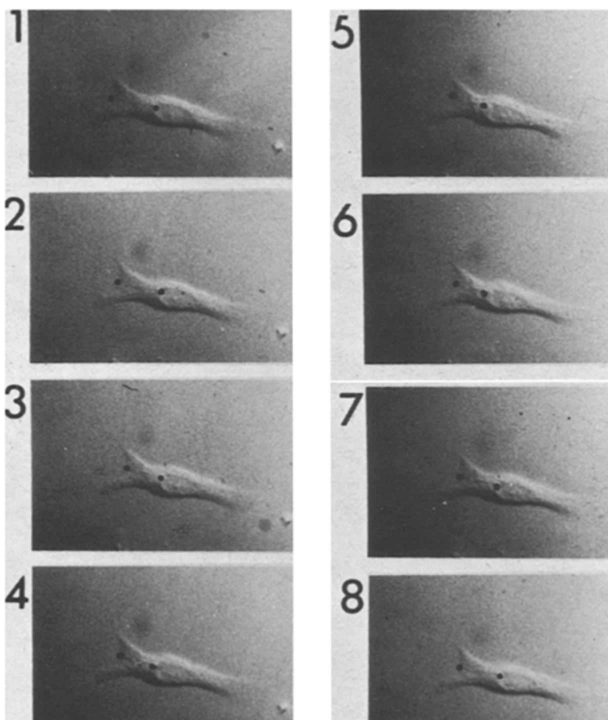

Fig. 1. Sequential Nomarski cinemicrographic frames show 1 cycle of chick vascular muscle contraction. The contraction began at frame 2, reached maximum shortening at frame 5, and relaxed nearly completely in frame 8 (the complete cycle occurred in 9 frames). The shortening area is highlighted by 2 black dots on the edge of the nucleus and the cell border. Notice that the interdot distance is shorter in frame 5 than in frame 1. The total length of the cell was 50 µm and did not change during contraction because the end points were attached to the glass coverslip. All of the movement was in the contractile proteins and cytoskeleton, and is seen by a widening of the cell in the region between the dots and a convergence of the dots. These contractions demonstrate the intracellular movement that occurs during an isometric contraction at the cell level. At the filming speed of 54 frames per sec, the interval between frames is 18.5 msec and total contraction duration was 167 msec. This is the shortest contraction cycle filmed.

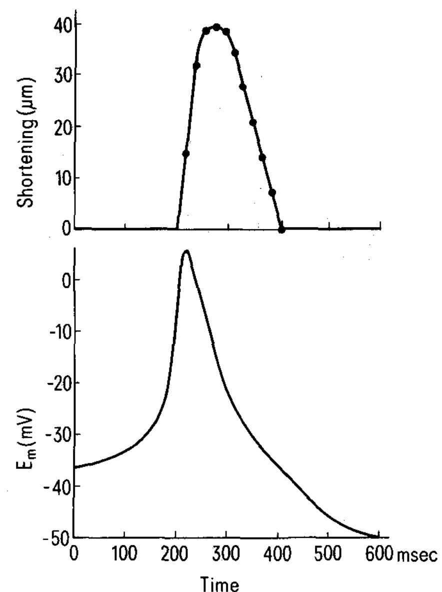

Fig. 2. A typical 200-msec contraction record taken from (projected) single frame film analysis and spontaneous membrane excitation underlying it. Total length of this synchronously contracting small cell group was 440 µm. Contractile time course and the spike duration at −30 mV were nearly equal. Contraction always began during the steep rising phase of the spike. Contractions of the S type were about 4 sec in duration caused by spontaneous slow depolarization of 10–20 mV, and were superimposable with the shortening curve.

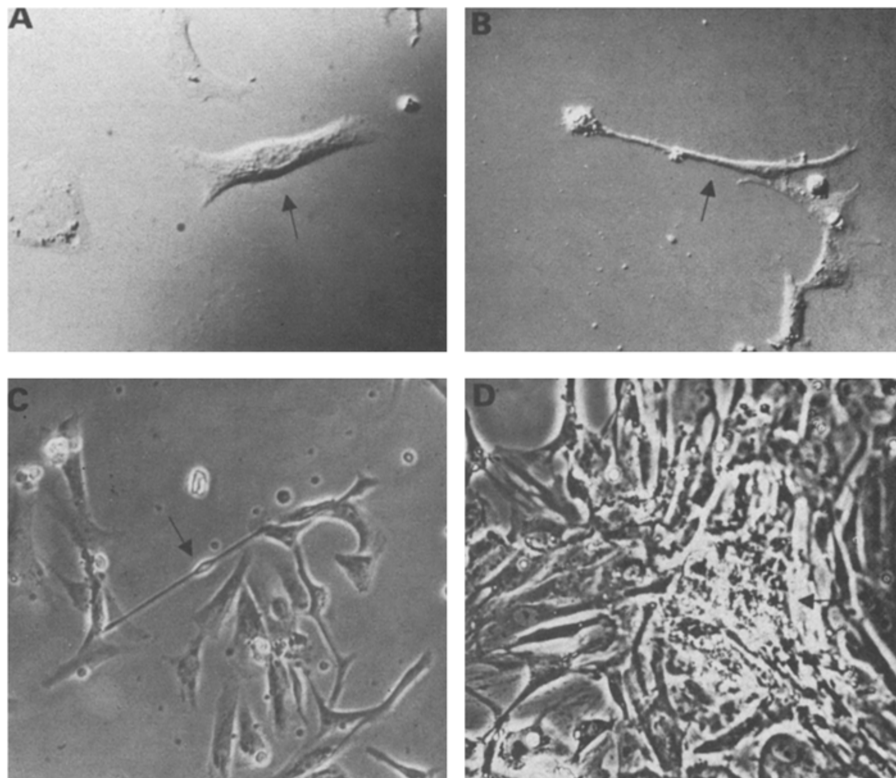

Fig. 3. Spontaneously contracting vascular muscle cells that showed high shortening velocity photographed during contraction. *A* A single contracting cell from rat aorta (×940 Nomarski). *B* A group of cells from rat aorta organized into a strand that showed independent contractions in 3 areas (×380 Nomarski). *C* A group of cells from chick aorta that contained a single contracting cell with long processes (×380 phase contrast). *D* The largest multi-layered group of rat vascular muscle cells to show high shortening velocity. The center refractile (lighter) portion contracted synchronously (×380 phase contrast). The contracting cell or cell group in each of these photomicrographs is marked with an arrow.

proliferation phases[19]. No membrane spikes of altered shape[20] were encountered in the present experiments. The change in the excitation mechanism is not likely to be due to trypsinization alone because trypsinization fails to alter isolated ventricle[15] or vascular muscle (unpublished observation). Since the cells reported in the present experiments converted to rapidly contracting cells within days, at which time contractile ability and pharmacological sensitivities were at least as great as in freshly isolated blood vessels, it appears likely that the conversion of membrane excitation is a fundamental part of the conversion to the high shortening velocity seen in the F contractions. However, other components contributing to such high shortening velocity such as contractile protein changes cannot be excluded by these experiments,

Because of the very rapid contractions observed in these cultures, I considered the possibility that the cultures could contain cardiac muscle. The 2 factors used to differentiate between cardiac and vascular muscle were the shape of the membrane electrical spike and the adrenergic receptor type and response. As was shown in figure 2, the spike in the cultured aortic muscle cells was absolutely free of any sign of a plateau phase. The cells thus appeared to be vascular muscle because there are distinct plateau phases, or at least an inflection on the falling phase, in cultured myocardial cells[15,16]. Pharmacologically, the cultured cells also appeared to be vascular muscle. When the β-adrenergic agonist isoproterenol was added to the quickly contracting cultures, none of the cells responded with a change in contraction time course or frequency. Concentrations of 0.1, 1, and 10 μM isoproterenol were inactive on contracting arterial muscle cells. However, the α-adrenergic agonist phenylephrine (0.1 μM) doubled the spontaneous contraction frequency of F cells and increased the spontaneous contraction frequency and duration in S cells. Both F and S types of arterial muscle cells thus appeared to retain their α-excitatory behavior. In contrast, isoproterenol increased the spontaneous contraction frequency of cardiac muscle cells 2.5 times. Therefore, in addition to the dissection of the thoracic aorta which meticulously avoided the heart, electrophysiological and pharmacological criteria indicate the vascular muscle identity of the quickly contracting cells. The stimulation of the cultured aortic muscle cells by α- but not β-adrenergic agents is similar to what is found in myogenically active hepatic portal vein[17].

As pointed out by Bozler[21] in 1948, the functional classification of muscles according to the presence or absence of striations is inappropriate, because the influences of innervation and membrane electrical properties are predominant in both striated and nonstriated cells. Bozler's terms multiunit, for organs like skeletal muscles and blood vessels with functionally independent parts under nervous control, and unitary, for organs behaving as a single all-or-none unit like heart and viscera, have been replaced in blood vessels by the terms nonspiking (gradedly depolarized) for arterial muscle and spiking for certain veins to emphasize the membrane activation mechanism[9]. Although the graded depolarization mechanism of arteries would imply that arterial muscle contractions should be quite slow, quick contractions might also be expected if spiking were to occur. In fact, contraction time courses similar to those found in skeletal muscle have been demonstrated in nonstriated muscle of *Beroe*[22]. Bozler's implication that under proper excitatory conditions quick phasic contractions might be possible in arterial muscle seems to have been demonstrated by the observations reported here.

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5 For preparation of the cell cultures, 10–12 embryos or neonates were opened under aseptic conditions and the portion of the thoracic aorta ≥ 2 mm distal to the aortic arch was removed and placed in CV2M medium consisting of 40% M199, 45% Earle's balanced salt solution, and 15% horse serum, to which 100 μg/ml gentamicin, 50 units/ml of penicillin, and 50 μg/ml streptomycin were added. After the tissue was minced into 1 mm cubes, the CV2M solution was poured off and 15 ml of Earle's balanced salt solution containing 3 mg/ml of collagenase (Worthington type III) were added. After 60 min of incubation at 37 °C without stirring, the collagenase solution was poured off and 15 ml of 1 mg/ml trypsin (1:250, ICN Pharmaceuticals, Inc.) in calcium, magnesium-free salt solution consisting of (in mM/l): 133 NaCl, 4.7 KCl, 20 HEPES buffer, 16.5 glucose and 0.014 phenol red, the final pH of which was 7.2, were added. The cells in trypsin solution were placed on a magnetic stirrer at 37 °C. The cells freed by the enzyme were poured off and more trypsin solution was added. 4 such treatments of 15 min each were used to essentially disperse the tissue fragments completely. After each 15 min dispersion, freed cells were placed in 50 ml centrifuge tubes, 5 ml of horse serum were added, and the tubes were kept in ice until all dispersion were complete. The cells were then centrifuged at 200 × g for 8 min. The supernatant was discarded, and 20 ml of the bathing solution were added to resuspend the cells and wash out traces of enzyme. This wash step was then repeated and the resulting pellet was diluted in 5 ml of warm growth medium, which consisted of CV2M with 2 mM 1-glutamine and 20 μg/ml of gentamicin added. The final dilution to a cell concentration of 0.5×10^6 cells per 5 ml culture was placed in plastic dishes containing a collagen-coated glass coverslip. The culture dishes were placed in a wet incubator at 37 °C and a 5% CO_2 atmosphere. Some of the cultures were prepared using filtration through a Swinny filter, as previously described (K. Hermsmeyer, P. DeCino and R. White, In Vitro *12*, 628 (1976)), which produces aggregates of cells in addition to cell sheets.
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8 For simultaneous recording of action potentials and contraction, electrophysiological data were collected by intracellular microelectrodes consisting of KCl-filled glass micropipettes connected to a W-P Instruments model 701 preamplifier through Ag:AgCl half-cells while cell contractions were recorded on cine film. The cells were recorded by microelectrodes under direct observation on the stage of a Leitz Diavert microscope equipped with a Zernike phase-contrast or Nomarski differential interference contrast optical system, and with a thermoregulated stage maintaining temperature at 37 °C, which was constructed by Jim's Instrument Manufacturing, Coralville, Iowa. Cells were observed in a 300 μl chamber during continuous suffusion with 50% M199 and 50% Earle's balanced salt solution. Contractions of the same muscle cells were recorded by cine micrography with a Leicina Special camera at up to 54 frames/sec and analyzed sequentially with a single frame advance projector (image size 45 × 60 cm). Movement of the contractile apparatus can be most easily observed by its movement of cell surface features as the contractile apparatus pulls its attachment points toward each other and widens the central part of the cell. Movements of the contractile apparatus were thus taken between distant points, and the change in length expressed as a fraction of the total cell length. Since the cell has attached itself to the coverslip, these contractions are essentially isometric. These contractile apparatus movements are analogous to the sliding filaments in skeletal muscle, but differ because the cytoskeleton filament attachment points allow much more movement than in skeletal muscle.
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Hepatic blood flow in acute myocardial ischemia[1]

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Summary. Hepatic blood flow was monitored in cats during myocardial ischemia (MI). Increased plasma CPK activity, the S-T segment of the electrocardiogram, and hepatic flow was reduced by 5 h to 40% of control. The results suggest that MI can influence organs distant from the original ischemic episode.

Myocardial ischemia (MI) is a complex disease entity involving a variety of cardiac and extracardiac phenomena[3]. Information regarding the cardiac processes responsible for the spread of ischemic damage in acute myocardial ischemia is becoming better understood[4]. However, extracardiac processes involved in the pathogenesis of acute myocardial ischemia remain poorly understood. The purpose of this study was to determine if myocardial ischemia, uncomplicated by cardiogenic shock, alters liver blood flow. If liver blood flow is compromised it could induce tissue injury resulting in a deficit in energy metabolism, as well as an impairment in phagocytosis of the reticuloendothelial system.

Methods. Male cats (3.8–5.3 kg) were anesthetized with