Evidence for absence of latch-bridge formation in muscular saphenous arteries

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Submitted 12 September 2005; accepted in final form 31 January 2006

Evidence for absence of latch-bridge formation in muscular saphenous arteries. Am J Physiol Heart Circ Physiol 291: H138–H146, 2006. First published February 3, 2006; doi:10.1152/ajpheart.00977.2005.—Large-diameter elastic arteries can produce strong contractions indefinitely at a high-energy economy by the formation of latch bridges. Whether downstream blood vessels also use latch bridges remains unknown. The zero-pressure medial thickness and lumen diameter of rabbit saphenous artery (SA), a muscular branch of the elastic femoral artery (FA), were, respectively, approximately twofold and half-fold that of the FA. In isolated FA and SA rings, KCl rapidly (<16 s) caused strong increases in isometric stress (1.2 × 105 N/m2) and intracellular Ca2+ concentration ([Ca2+]i; 250 nM). By 10 min, [Ca2+]i declined to approximately 175 nM in both tissues, but stress was sustained in FA (1.3 × 105 N/m2) and reduced by 40% in SA (0.8 × 105 N/m2). Reduced tonic stress correlated with reduced myosin light chain (MLC) phosphorylation in SA (28 vs. 42% in FA), and simulations with the use of the four-state kinetic latch-bridge model supported the hypothesis that latch-bridge formation in FA, but not SA, permitted maintenance of high stress values at steady state. SA expressed more MLC phosphatase than FA, and permeabilized SA relaxed more rapidly than FA, suggesting that MLC phosphatase activity was greater in SA than in FA. The ratio of fast-to-slow myosin isoforms was greater for SA than for FA, and on quick release, SA redeveloped isometric force faster than FA. These data support the hypothesis that maintained isometric force was 40% less in SA than in FA because expressed motor proteins in SA do not support latch-bridge formation.

Vascular smooth muscle; myosin light chain phosphorylation; KCl; myosin isoforms; calcium

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A latch bridge is absent for force maintenance in muscular arteries as it is in direct arterioles and atriope (34). The optimum muscle length ($L_o$) for which active force was maximum was determined for each tissue by using an abbreviated length-tension curve and PSS in which 110 mM KCl was substituted isosmotically for NaCl (KPSS) as the stimulus (17, 32). To eliminate effects of norepinephrine and acetylcholine released from nerves, 1 $\mu$M phenolamine and atropine were used to block, respectively, $\alpha_1$-adrenergic and muscarinic receptors in arteries and detrusor. Muscle stress (S) in N/m$^2$ was calculated as follows: $F/[f \times 9.807 \times 10^{-3} \text{N/g}] \times [\text{wt wt (in mg)}/L_o \text{ (in mm)}] \times 9.52 \times 10^{-7} \text{m}^2/\text{mm/g}$.

Tissue histology. To measure medial wall thickness and numbers of cell layers in the media, arterial rings were fixed (glutaraldehyde), embedded (PolyBed 812 resin), and sectioned with an ultramicrotome, transferred to a microscope slide, and stained with 0.1% Toluidine blue/0.1% methylene blue/0.1% azure II in 1% sodium borate solution (22). Wall thickness of each tissue was measured, and the numbers of cell layers for each cross section were counted with a microscope (Olympus IX71) and OpenLab software (Improvement). Polyacrylamide gel electrophoresis. Two-dimensional (isoelectric focusing × SDS-PAGE) was performed as previously described (34, 47) to measure the degree of MLC phosphorylation and the fractional expression of the 17-kDa MLC isoforms MLC$_{17a}$ and MLC$_{17b}$. Expression levels of MLC phosphatase catalytic subunit (PP1), MLC-PAGE phophatase large molecular weight regulatory subunit (MYPT-1), and RhoA kinase $\alpha$ (ROK$\alpha$) were measured by SDS-PAGE and Western blot analysis as described previously (35). Protein loading was verified to be constant across all lanes by MEMCode (Pierce) staining. Dilutions of primary antibodies were 1:500 for anti-PP1 (Upstate) and anti-MYPT-1 (BD Transduction) and 1:200 for anti-ROK$\alpha$ (Santa Cruz Biotechnology). Horseradish peroxidase-conjugated goat polyclonal antibody was used as secondary antibody, and the amounts of specific protein were detected by enhanced chemiluminescence (Amersham) and quantified after digitization by Scion Image Software. For MHC isoform expression analyses, rabbit tissues were homogenized in 0.125 M Tris, 2% SDS (wt/vol), 20% glycerol, 0.1% bromophenol blue (wt/vol), and 20 mM dithiothreitol. MHCs were resolved on low cross-linking SDS gels (11), and immunoblotting was performed as previously described (8). Polyclonal antibodies to the SMB (plus seven-amino acid head insert isoform) and SMA (minus seven-amino acid head insert) smooth muscle MHC isoforms were generated in rabbits by using the following peptides: (N‘-KBDTSITGELEC-C‘; SMB polypeptide- N‘-QGPSLAYGELEC-C‘). Antiserum was tested on ELISA against expressed SMA and SMB subfragment 1 polypeptides. Both antibodies were shown to have at least 100-fold higher affinity for their appropriate antigen than for the alternative isoforms. Smooth muscle and nonmuscle MHC-specific antisera were obtained from Biomedical Technologies (Stoughton, MA). Western immunoblots were reacted as reported previously (10).

[$Ca^{2+}$]$_i$, was measured as previously described (34). Tissues at $L_o$ in an aerated muscle chamber designed for microscopic imaging (Danish Myo Technology) were placed on the stage of an inverted microscope (Olympus IX71) and loaded for 2.5 h with 7.5 $\mu$M fura 2-PE3 (AM) and 0.01% (wt/vol) Pluronic F-127 (Tel Labs, Austin, TX) to enhance solubility. Fluorescence emission at 510 nm was collected by a photomultiplier tube for excitations at 340 and 380 nm (DeltaRam V, Photon Technologies, Lawrenceville, NJ), and emission intensities were expressed as 340/380 nm ratios with the use of Felix software (Photon Technology International) to measure changes in [$Ca^{2+}$]$_i$. Minimum and maximum fluorescence ratios were obtained by treating tissues with, respectively, a $Ca^{2+}$-free KPSS containing 5 mM EGTA and 30 $\mu$M ionomycin and a high-calcium (3.2 mM Ca$^{2+}$) KPSS containing 30 $\mu$M ionomycin. Background fluorescence, determined by incubating tissues in 4 mM MnCl$_2$ plus 30 $\mu$M ionomycin, was subtracted from all 340 and 380 nm signals before calculation of the 340/380 nm fluorescence ratios. [$Ca^{2+}$]$_i$, was calculated as described previously (13).


**Tissue permeabilization.** Artery rings at L_{o} were depleted of sarcoplasmic reticular Ca^{2+} by contracting three times with 10 μM phenylephrine in a Ca^{2+}-free solution. Tissues were then permeabilized at 5°C for 45 min with β-escin (40 μM for FA and 100 μM for SA; the higher concentration for SA was used because of its thicker media), and permeabilization continued for 60 min at 30°C. The initial treatment with β-escin at a low temperature helps the slow penetration and/or binding of β-escin to the surface membrane of the smooth muscle cells (23). β-Escin was dissolved in a “relaxing solution” containing 74.1 mM potassium methanesulfonate, 4.0 mM magnesium methanesulfonate, 4 mM Na_{2}ATP, 4 mM EGTA, 5 mM creatine phosphate, and 30 mM PIPES and neutralized with 1 M KOH to pH 7.1 at 20°C. Ionic strength was kept constant at 0.18 M by adjusting the concentration of potassium methanesulfonate. To activate muscle contraction at Ca^{2+}-clamped levels of 1 μM (pCa = 6) free Ca^{2+}, a “contracting solution” was made by including the appropriate volume of a 1 M CaCl_{2} stock (Fluka Chemicals) as determined by using WEBMAXC (30). Calmodulin (1 μM) was added to the solutions throughout each experiment to compensate for its loss during permeabilization. To induce relaxation for relaxation velocity measurements, tissues precontracted by pCa = 6 contracting solution were rapidly washed in the relaxing solution (pCa = 9) containing 3 μM wortmannin to inhibit MLC kinase activity.

**Latch-bridge model simulation.** The Hai-Murphy kinetic four-state latch-bridge model (Fig. 1) was simulated with the use of Matlab 6.5 with Simulink 5.0 (MathWorks) by using the same initial conditions and assumptions as those used by Hai and Murphy (15). Because Matlab 6.5 permits the use of time-varying constants, this feature was employed for rate constants simulating changes in MLC kinase and phosphatase activities to more closely reflect the time-varying changes in [Ca^{2+}i], and, thus, MLC kinase activity and regulation of MLC phosphatase by ROK [see review by Ratz et al. (37)]. We used modeling rate constants (in s^{-1}) that were very similar to those used by Hai and Murphy to predict the behavior of the tonic swine carotid artery (15). However, rather than stepping K_{1} and K_{0} values from 0 to 0.55 for 5 s and then down to 0.30 for the remainder of the simulated stimulation period to mimic changes in MLC kinase activity due to changes in [Ca^{2+}i] (15), we used similar values but modeled a more gradual change to more closely follow the change in [Ca^{2+}i] measured in SA and FA (see Fig. 6, C and D). We also modeled a small temporal change in K_{2} and K_{5} values to reflect recent data (37) indicating that KCl can activate ROK and produce a transient small increase in MYPT-1 phosphorylation that may transiently reduce MLC kinase activity (see Fig. 6, E and F).

**Drugs.** Wortmannin, Y-27632, and ionomycin were from Calbiochem (La Jolla, CA), and all other drugs were from Sigma (St. Louis, MO). β-Escin was dissolved in DMSO for a stock concentration of 10 mM. Ionomycin was dissolved in 100% ethanol for stock solutions of 10 mM. Vehicles (DMSO and ethanol) were added to control tissues at no more than 0.1%, which had no effect on contractions. All other drugs were dissolved in distilled water.

**Statistics.** The null hypothesis was examined with the use of Student’s t-test (when 2 groups were compared) or a one-way ANOVA. To determine differences between groups following ANOVA, the Student-Newman-Keuls post hoc test was used. In all cases, the null hypothesis was rejected at P < 0.05. For each study described, the n value was equal to the number of rabbits from which arteries were taken. Statistical analyses and curve fitting were performed with the use of Prism 3.02 (GraphPad Software, San Diego, CA).

**RESULTS**

**Structure.** Of the two major branch arteries that bifurcate from the femoral artery (FA) just cranial to the knee, only the deep femoral (DF) artery grossly (Fig. 2Aa) and histologically (Fig. 2, Ab–Ad) represented an extension of the FA, whereas the SA was structurally different from the FA. For example, the FA and DF artery had equivalent medial thicknesses (Fig. 2B) and numbers of cell layers within the media (Fig. 2C) and nearly equivalent L_{o} for muscle contraction (Fig. 2D), a muscle mechanical measurement that reflects artery lumen diameter. However, compared with FA, SA media were approximately twofold thicker (Fig. 2B) because of approximately twofold more medial cell layers (Fig. 2C) but displayed a shorter L_{o} (D) correlating with a smaller lumen diameter (E) than the FA. Data in B–E are means ± SE and n = 3, except for D, where n = 13, and E, where n = 4 (FA) and 9 (SA). *P < 0.05 compared with FA.

**Stress, [Ca^{2+}i], and MLC phosphorylation.** KCl produced tonic contractions in the FA (Figs. 3, A and B) and DF artery (Fig. 3B) with equivalent time-dependent force values. By contrast, SA produced a biphasic contraction in response to stimulation with KCl consisting of an early peak increase in stress equal to the maximum tonic stress produced by FA followed by a decline from the peak value by 5 and 10 min to a value ∼60% that produced by FA (Fig. 3A; P < 0.05). A maximum phenylephrine concentration (10 μM), like KCl, also produced tonic and biphasic contractions in, respectively, FA and SA (data not shown). Despite divergent temporal changes in stress, FA and SA produced equivalent temporal changes in [Ca^{2+}i], when stimulated with KCl (Fig. 3, C and D). Thus the difference in steady-state stress between FA and SA could not be ascribed to a difference in [Ca^{2+}i].

KCl produced a rapid increase in the degree of MLC phosphorylation from a basal value of ∼5–40% within 4 s in both FA and SA (Fig. 3E). However, MLC phosphorylation...
continued to increase to over 60% by 10 s in FA but remained at ~40% in SA, a value statistically lower than that produced by FA (Fig. 3E). MLC phosphorylation declined in both FA and SA to lower steady-state values. However, SA reached a steady-state MLC phosphorylation value of ~28% within 30 s and remained at that level for at least 10 min, whereas FA produced a more gradual decline to a steady-state value of ~42% at 10 min (Fig. 3, E and F). The correlation in MLC phosphorylation and stress values (Fig. 3F) suggests that the biphasic nature of the KCl-induced contraction produced by SA (in which the level of tonic force was less than that produced by FA) was caused by the reduced level of steady-state MLC phosphorylation in SA compared with FA.

**Estimate of MLC phosphatase activity and expression of MLC phosphatase.** The rate of relaxation of permeabilized arterial smooth muscle is limited by the rate of MLC phosphatase activity (25). Thus the rate of relaxation from a precontracted state induced by exposure of permeabilized tissues to a Ca$^{2+}$-free solution containing a MLC kinase inhibitor can be used as an indirect measure of the MLC phosphatase activity in Ca$^{2+}$-clamped but otherwise intact smooth muscle tissue (21). Our data showed that SA produced a much more rapid relaxation compared with FA when tissues precontracted at pCa = 6 were exposed to a relaxing solution (containing EGTA; see methods) and the MLC kinase inhibitor wortmannin (Fig. 4A). The half-time for relaxation was nearly 2 min in FA but was <1 min in SA (inset, Fig. 4A). To ensure that relaxation rates reflected rates of MLC dephosphorylation in both FA and SA, MLC phosphorylation and force were measured simultaneously in one set of tissues. The nearly linear relationship between MLC phosphorylation and force (see inset, Fig. 4B) supports the conclusion that relaxation rates in permeabilized tissues can be a surrogate measure of MLC dephosphorylation and reflect MLC phosphatase activity (21, 25). These data suggest that the lower MLC phosphorylation levels produced in intact (not permeabilized) SA compared with FA during a KCl-induced contraction are caused by a higher intrinsic MLC phosphatase activity in SA compared with FA.

We compared, in FA and SA, expressed levels of MLC phosphatase catalytic (PP1), or different roles in SA and FA, intact tissues were estimated to curves in C and included in D; n = 4–10. *P < 0.05 compared with FA.
KCl-induced steady-state (tonic phase) contractions of SA and FA.

Latch-bridge model. The Hai-Murphy four-state kinetic latch-bridge model (see Fig. 1) was employed to determine whether the reduction in MLC phosphorylation in SA compared with FA was sufficient to explain the biphasic contractile behavior of SA. With a $K_4$ value of 0.05, a $K_3$ value of 0.4, and, as in the original Hai-Murphy model (15), a $K_7$ (latch bridge) value one-tenth that of the $K_4$ value ($K_7 = 0.005$), the modeled temporal change in MLC phosphorylation fit closely the empirically derived MLC phosphorylation data for FA (Fig. 6G). Our data showing that steady-state MLC phosphorylation (see Fig. 3, E and F), but not $[Ca^{2+}]_i$ (see Fig. 3, C and D), was less in SA than FA suggested that MLC phosphatase in SA was greater than that in FA. Thus $K_2$ and $K_5$ values used to model FA behavior were doubled to model SA behavior (Fig. 6F). The resulting modeled change in MLC phosphorylation fairly closely matched the empirical MLC phosphorylation data for SA (Fig. 6H).

The force response predicted on the basis of a latch-bridge-to-phosphorylated cross-bridge detachment ratio ($K_7/K_4$) of 0.1 very closely matched our empirical data for FA (Fig. 6A, model L$_{FA}$) but was a poor match for SA (Fig. 6B, model L$_{SA}$). When the detachment rate for a latch bridge was made to equal the detachment rate for a phosphorylated cross bridge (i.e., $K_7/K_4 = 1$), the modeled force profile more closely fit the actual empirical data for SA (Fig. 6B, model NL$_{SA}$).

Because of the steeply hyperbolic dependency of steady-state force on steady-state MLC phosphorylation in elastic arteries such as the carotid artery, where force “saturates” at modest levels of MLC phosphorylation (33, 34), a reduction in MLC phosphorylation from over 40% to just under 30% will not necessarily result in a significant reduction in force. Rather, total force will remain relatively constant as the fraction of phosphorylated, attached cross bridges (AMP) is reduced and the fraction of unphosphorylated, attached cross bridges (AM) is proportionally increased. That is, the latch bridge-modeled force profiles for FA (Fig. 7A, AMP + AM, dashed line) and SA (Fig. 7A, AMP + AM, solid line) were found to be nearly equivalent despite large differences in MLC phosphorylation values because the proportion of cross bridges contributing to steady-state (5 min) force for the FA was ~60% latch bridge (Fig. 7A, AM, dashed line) and ~40% phosphorylated, attached, cycling cross bridge (Fig. 7A, AMP, dashed line), whereas that for SA was ~73% latch bridge (Fig. 7A, AM, solid line) and ~27% phosphorylated, attached, cycling cross bridge (Fig. 7A, AMP, solid line). Thus the reason why a reduction in the level of MLC phosphorylation from ~42% to ~28% did not result in a comparable reduction in force in the four-state kinetic simulation has a straightforward and readily predictable explanation based on the Hai-Murphy latch-bridge model.

Elimination of the latch bridge (without eliminating the myosin species, AM) by making $K_4$ equal $K_7$ (no latch; NL$_{SA}$, Fig. 7B; see also Fig. 1) caused force to fall temporally with the fall in MLC phosphorylation (i.e., force became biphasic, Fig. 4).
rates of force redevelopment on quick release. steady-state levels of MLC phosphorylation correlate with steady-state levels of the velocity of muscle shortening (33), and the rate of force redevelopment on quick release (Fig. 8A) is a measure of the velocity of muscle shortening (6). the proposed reason for a correlation between MLC phosphorylation levels and the rate of force redevelopment is that the ratio of cycling cross bridges to latch bridges (AMp/AM) is higher at higher levels of MLC phosphorylation (Fig. 5 in ref. 15; see also Fig. 7A for an example). latch bridges are proposed to introduce an internal load against which cycling cross bridges operate, causing slowing of the rate of muscle shortening. thus, if SA contains latch bridges, then SA should recontract more slowly than FA on quick release at ~10 min of contraction because SA produced significantly lower MLC phosphorylation levels at this time (see Fig. 3, E and F). our empirical data revealed the opposite, namely, that the rate of force redevelopment produced by SA was significantly higher than that for FA (Fig. 8). With the use of a curve-fitting program (GraphPad Prism), the rate of force redevelopment for both arteries produced a good

Fig. 7. Hai-Murphy 4-state kinetic latch-bridge model simulations for FA and SA comparing time-dependent changes in total force (AMp + AM) and two force-producing species (attached, phosphorylated cross bridges, AMp, and attached, unphosphorylated cross bridges, AM). When compared with the relatively low level of latch-bridge species formed (A, dashed line, AM) at a relatively high level of simulated MLC phosphorylation (A, dashed line, AMp), mimicking empirical data from FA, a lower level of simulated MLC phosphorylation (A, solid line, AMp) that mimics level found to occur in SA yields a higher simulated level of latch-bridge species (A, solid line, AM). Thus, despite a lower level of MLC phosphorylation in SA, latch-bridge formation would disallow a temporal fall in tonic force, i.e., biphasic contractile behavior seen empirically in SA (AMp + AM = force). Absence of latch-bridge state (B, NLSA, k7 = k4) in simulation permits a temporal fall in force, resulting in a more biphasic contraction (B, solid line compared with dashed line, AMp + AM) that more closely mimics empirical temporal force profile produced in SA on KCl-induced stimulation.

Fig. 8. A: hyperbolic + linear curve provides a good modeling fit (r2 > 0.97) for force redevelopment after quick, 10% step decrease in muscle length (inset; QR, quick release) during steady state of KCl-induced contraction for both FA and SA. B: half-lives (t1/2) of hyperbolic component and slopes (m) of linear component of curves for FA and SA and individual hyperbolic and linear curve; n = 4.
fit ($r^2 = 0.996$ for FA; $r^2 = 0.974$ for SA) to an equation consisting of one fast hyperbolic and one slower linear component (Fig. 8A). The half-time for force redevelopment for the hyperbolic component of FA was over sevenfold longer than that for SA (i.e., the rate of force redevelopment for SA was over sevenfold faster than FA; Fig. 8B), and the slope of the slower, linear component for SA was threefold faster than for FA ($m_{SA}$ and $m_{FA}$ in Fig. 8B). These data together support the hypothesis that FA does, but SA does not, form latch bridges to maintain steady-state force.

**Smooth muscle motor protein expression levels.** Higher rates of force redevelopment for SA compared with FA support the hypothesis that SA contains “faster” motor protein isoforms than FA [see Arner et al. (2) for a review]. To test this hypothesis, the relative expression of MLC17a and MLC17b were examined using two-dimensional PAGE, and smooth muscle MHC isoforms that do (SMB) and do not (SMA) contain a seven-amino acid insert were examined by using Western blot analysis and selective antibodies. The FA, DF artery, and tonic renal artery (RA) displayed ~50–60% MLC17a, whereas SA and a classically phasic visceral smooth muscle (detrusor) displayed 90–100% MLC17a (Fig. 9A). The tonic arteries, aorta, carotid artery, FA and RA, and a visceral smooth muscle known to produce sustained, tonic contraction, the stomach fundus, displayed higher relative expression levels of SMA compared with SMB, whereas SA and a phasic visceral smooth muscle (known to contract only transiently and not maintain high tonic force levels), the stomach antrum, displayed higher relative levels of SMB compared with SMA (Fig. 9, Bd and Be). None of the smooth muscles examined expressed enough nonmuscle myosin to be detected when compared with an equal protein loading of platelets that express nonmuscle rather than smooth muscle myosin (Fig. 9Bc). These data support the notion that SA produced faster force redevelopment than FA despite lower MLC phosphorylation levels at steady state because SA expressed quantitatively more of the “faster” (SMB and MLC17b) compared with “slower” (SMA and MLC17a) myosin isoforms (2).

**Selective proteomic analysis.** On the basis of its temporal contractile profile, rate of force redevelopment, and motor protein isoform composition, the SA may be categorized along with visceral smooth muscles represented by detrusor and stomach antrum as a “phasic” smooth muscle because these muscles produce “fast” contractions and do not produce strong, sustained force during the tonic phase of contraction. Thus the contractile phenotype and the motor protein expression of SA are more similar to that of most visceral smooth muscles than to its “parent” elastic arterial smooth muscle, the FA. Regulation of smooth muscle contraction requires the interaction of many complex signaling, metabolic, and structural systems, and whether some arterial muscles are more closely related overall to visceral smooth muscles or to each other is an important question that remains to be determined. To examine whether the general protein expression profile of SA is more like that of a phasic visceral smooth muscle or a tonic arterial smooth muscle, an initial proteomic analysis was performed.

The abundance of highly expressed proteins relative to actin in FA, SA, and detrusor were compared by using one-dimensional PAGE (Fig. 9C). Of the nine abundantly expressed proteins readily identified by Coomassie blue staining that likely participate in contraction (48), three (filamin, MHC, and desmin) were expressed in greater abundance in the detrusor compared with FA and SA (Fig. 9D). Interestingly, desmin expressed less calponin than SA ($P < 0.05$; Fig. 9D). Only 22-kDa smooth muscle-specific protein was expressed in increased abundance in the FA compared with SA and detrusor ($P < 0.05$; Fig. 9D), and no other of the nine proteins displayed differential expression when comparing SA to FA (Fig. 9D). These data together suggest that, overall, FA and SA were more similar than the SA and detrusor, despite the finding that, when considering motor protein expression, the SA and detrusor were more similar than SA and FA.

**DISCUSSION**

Results from the present study support the hypothesis that MLC phosphorylation and differential motor protein isoform expression determine the degree of developed sustained (tonic) isometric force in arterial smooth muscle. The elastic FA...
expressed “slow” myosin isoforms and maintained relatively high KCl-induced levels of tonic MLC phosphorylation and force on stimulation with KCl, whereas the muscular SA expressed “fast” myosin isoforms and produced considerably weaker KCl-induced tonic levels of MLC phosphorylation and force. The most important finding was that SA, unlike its “parent” FA, did not appear to enter the latch state.

The mechanism of tonic force maintenance in the FA was very similar to that described for the carotid artery, another large elastic artery (5, 15, 33, 38). That is, maintenance of strong tonic forces in the face of falling levels of [Ca^{2+}], and MLC phosphorylation in both FA and carotid artery can be ascribed to the formation of latch bridges. Latch bridges are proposed to impose an internal load against which rapidly cycling cross bridges must act (7), so the “trade-off” for permitting high force maintenance at a high energy economy [see review by Murphy (27)] is a reduced rate of muscle shortening (5, 7, 33). Whether latch bridges reflect an adaptation developed specifically to address the physiological requirements of all VSM or only of specific vascular segments is not known. However, visceral smooth muscles, such as the urinary bladder detrusor and stomach antrum that contract quickly and do not maintain high levels of tonic force, express “fast” myosin isoforms and display rapid actomyosin detachment rates, reflecting a lack of latch-bridge formation (9, 19, 46). We propose that low steady-state MLC phosphorylation levels and apparent absence of latch-bridge formation in the SA precluded its ability to maintain high levels of isometric force for the duration of a KCl-induced stimulation period.

The latch state provides a molecular explanation for maintenance of high VSM isometric stress in the face of falling levels of [Ca^{2+}], MLC phosphorylation, and the maximum rate of cross-bridge cycling [see Dillon et al. (5), Dillon and Murphy (7), Hai and Murphy (15), and Murphy (27) for reviews]. As anticipated, a computer simulation of contraction based on the latch-bridge model demonstrated that MLC phosphorylation was not reduced enough by 5 and 10 min to permit a fall in stress in FA and SA. Rather, the latch-bridge model predicted that contraction should be maintained at high levels in SA and FA even when MLC phosphorylation fell from ∼40 to ∼28%. This is because a decrease in MLC phosphorylation from 40 to 20%, while reducing the fraction of phosphorylated, attached cross bridges, causes an increase in the fraction of dephosphorylated, attached cross bridges (i.e., latch bridges) (15). In fact, the signature feature of the latch-bridge model is that falling levels of MLC phosphorylation permit maintenance of high levels of force at a high energy economy by forming increased numbers of latch bridges [see Refs. 15 and 33 and also reviews by Murphy (27) and Murphy et al. (28)]. Thus the latch-bridge model predicts that, at steady state, SA should contain more latch bridges than FA because of a lower steady-state (tonic) level of MLC phosphorylation. However, if this were the case, then force redevelopment at steady state, a measure of cross-bridge cycling velocity (6), should be lower in SA than in FA, because a higher ratio of latch bridges-to-cycling (phosphorylated) cross bridges would impede cross-bridge cycling velocities (33). This was found not to be true. In fact, SA redeveloped force at a much higher rate than did FA, which is consistent with a report by Sherwood and Eddinger (43) that single smooth muscle cells isolated from SA shorten more rapidly than those isolated from FA. In short, the hypothesis that the motor protein isoforms of SA formed latch bridges is not supported by our data showing that steady-state force fell nearly by half over a 5-min period from initiation of a KCl stimulus and that SA displayed much higher apparent cross-bridge cycling rates despite much lower levels of MLC phosphorylation than FA.

The lower levels of MLC phosphorylation produced by SA compared with FA could not be explained by a lower level of [Ca^{2+}]. Also, our data using inhibitors of MLC kinase and ROK suggest that additional MLC phosphorylation regulatory systems need not be invoked to explain the differential MLC phosphorylation values comparing FA and SA. Smooth muscle MLC phosphatase catalytic subunit is a PP18 serine/threonine protein phosphatase isoform (20), and SA expressed nearly twofold more PP18 than did FA. Moreover, SA expressed ∼20% more MYPT-1, the regulatory subunit of MLC phosphatase. Thus one possible scenario is that, because of higher cellular levels of MLC phosphatase in SA compared with FA, the MLC phosphatase-to-kinase ratio was likewise elevated, reducing the steady-state level of MLC phosphorylation for a given [Ca^{2+}]. This hypothesis is supported by data obtained in permeabilized, Ca^{2+}-clamped tissues, suggesting that MLC phosphatase activity was approximately twofold greater in SA compared with FA.

There has been a great deal of speculation about the function of different MHC and MLC_{17} isoforms [see reviews by Arner et al. (2), Morano (26), and Ogut and Brozovich (29)], but a generally accepted model is that smooth muscles that display the phasic phenotype (fast contraction and weak tonic force maintenance) also display higher relative expression of “faster” myosin isoforms [i.e., SMB and MLC_{17a} compared with SMA and MLC_{17b}; see review by Arner et al. (2)]. Results from the present study support this hypothesis and provide evidence that expression of the “tonic” phenotype and latch-bridge formation is not a characteristic of all VSM. What remains to be determined is whether or not this hypothesis can be extended to blood vessels further down the vascular tree, or whether this conclusion is only valid for selected muscular arteries or selected vascular beds.

In conclusion, our data support a model whereby muscular arteries do not require expression of “slow” motor proteins and latch-bridge formation. Precisely why VSM of different segments of the vascular tree displays such dramatically different mechanical and regulatory behavior remains to be determined, but preliminary data from our laboratory (unpublished observations) support the view that latch bridges are an adaptation permitting constricted large-diameter arteries to temporarily resist full dilatation during increases in arterial pressures above the physiological resting level. Furthermore, these data support the speculative hypothesis that latch-bridge formation may not be a requirement of any blood vessels downstream from large elastic arteries, because of the absence of a physiological requirement to resist dilatation in blood vessels that normally can fully constrict to reduce luminal diameters even at very high pressures.

GRANTS

This work was supported by National Heart, Lung, and Blood Institute Grants R01-HL-61320 (to P. H. Ratz) and R01-HL-62237 (to T. J. Eddinger).
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