Phosphorylation events associated with cyclic nucleotide-dependent inhibition of smooth muscle contraction

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1Department of Cell Biology and Anatomy, 2Department of Surgery, 3Department of Medicine, Institute of Molecular Medicine and Genetics, and 4Department of Physiology and Endocrinology, Medical College of Georgia, Augusta 30912; and 5Augusta Veterans Affairs Medical Center, Augusta, Georgia 30901

Woodrum, David A., Colleen M. Brophy, Christopher J. Wingard, Arthur Beall, and Howard Rasmussen. Phosphorylation events associated with cyclic nucleotide-dependent inhibition of smooth muscle contraction. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H931–H939, 1999.—Activation of cyclic nucleotide-dependent signaling pathways leads to relaxation of bovine carotid artery smooth muscle contractions and is associated with increased phosphorylation of the small heat shock-related protein (HSP20). Previous reports have shown that human umbilical artery smooth muscle is uniquely resistant to cyclic nucleotide-dependent relaxation, and HSP20 is not phosphorylated. In this investigation, we determined the phosphorylation events associated with cyclic nucleotide-dependent inhibition of smooth muscle contraction. In carotid artery, activation of cyclic nucleotide-dependent signaling pathways inhibited contractile responses to serotonin but did not inhibit myosin light chain phosphorylation or oxygen consumption. The inhibition of contraction was associated with increases in HSP20 phosphorylation. In umbilical artery, activation of cyclic nucleotide-dependent signaling pathways did not inhibit serotonin-induced contraction or myosin light chain phosphorylation. The lack of contractile inhibition in umbilical artery was not associated with significant increases in HSP20 phosphorylation. In conclusion, cyclic nucleotide-dependent contractile inhibition is independent of the inhibition of myosin light chain phosphorylation or oxygen consumption but does correlate with increased HSP20 phosphorylation.

heat shock proteins; myosin light chains; oxygen consumption

IT HAS BEEN PROPOSED that smooth muscle contraction is initiated by increases in intracellular Ca2+ that activate myosin light chain kinase, leading to increases in the phosphorylation of myosin light chains (15, 19). The phosphorylated myosin light chains promote the interaction between actin and myosin, activate the myosin ATPase, and result in force generation (15, 18, 19). However, the maintenance of force in tonic muscle is associated with decreases in myosin light chain phosphorylation and intracellular Ca2+. To explain this behavior, a model describing a “latch state” proposed that dephosphorylated myosin light chains are associated with slowed cross-bridge cycling, decreased ATPase activity, and sustained force (14, 15). This model assumes that initiation and maintenance of smooth muscle contraction are modulated by changes in the phosphorylation state of smooth muscle myosin light chains (25).

Activation of smooth muscle contraction leads to the hydrolysis of ATP and the consumption of oxygen. Because of the relatively slow time course of smooth muscle contraction and the limited phosphocreatine and glycogen stores, there is a tight association between energy utilization and metabolic recovery (16). Under conditions in which oxygen and glucose are not limited, oxygen consumption directly correlates with the contractile state (28). In addition, oxygen consumption has been related to increases in the phosphorylation of myosin light chains and activation of myosin ATPase (40, 41). Therefore, energetic measurements can be used to evaluate the activation state of smooth muscle.

Vascular smooth muscle relaxation occurs in two ways: passive relaxation by removal of the contractile agent or active relaxation resulting from the activation of cyclic nucleotide-dependent signaling pathways in the continued presence of the contractile agent. AMP-dependent protein kinase and cGMP-dependent protein kinase are serine-threonine protein kinases that affect cellular processes by phosphorylating specific substrate proteins. Possible cyclic nucleotide-dependent protein kinase substrates include the inositol 1,4,5-trisphosphate (IP3) receptor, plasma membrane Ca2+ pump, phospholamban, myosin light chain kinase, and telokin. The phosphorylation of the IP3 receptor may lead to inhibition of IP3-induced Ca2+ release (21). The phosphorylation of the plasma membrane Ca2+ pump leads to increased affinity for Ca2+ (10). The phosphorylation of phospholamban decreases its interaction with the Ca2+-ATPase, thereby activating the Ca2+-ATPase (9, 30). Cyclic nucleotide-dependent phosphorylation of myosin light chain kinase inhibits its activity, resulting in decreased phosphorylation of myosin light chains (8). The phosphorylation of telokin activates myosin light chain phosphatase and decreases myosin light chain phosphorylation in visceral smooth muscle (42). Thus these putative cyclic nucleotide-dependent protein kinase substrates cause smooth muscle relaxation through decreases in myosin light chain phosphorylation or intracellular Ca2+.

On the other hand, several studies have shown that cyclic nucleotide-dependent activation uncouples force from myosin light chain phosphorylation (1, 11, 24). The decreases in active tension in forskolin-treated muscle are faster than the dephosphorylation of myosin.
light chains (1, 12, 24). Pretreatment with isoproterenol, forskolin, or 8-bromoadenosine 3',5'-cyclic monophosphate does not inhibit carbachol-induced increases in intracellular Ca$^{2+}$ concentration in bovine tracheal smooth muscle, yet these agents cause relaxation of the muscle (33). In addition, cyclic nucleotide analogs induce vasorelaxation in permeabilized smooth muscles when the Ca$^{2+}$ concentration is high and fixed (26). These data suggest that increases in intracellular cyclic nucleotide concentrations can lead to relaxation by mechanisms independent of changes in intracellular Ca$^{2+}$ concentrations or the state of myosin light chain phosphorylation.

The small heat shock-related protein (HSP20) has been identified as a cyclic nucleotide-dependent protein kinase substrate in vascular smooth muscle (4). Increases in the phosphorylation of HSP20 have been associated with cyclic nucleotide-dependent relaxation of bovine carotid artery and human peroneal artery smooth muscle (4, 5). However, increases in the phosphorylation of HSP20 have not been shown in human umbilical artery smooth muscle, a muscle that is uniquely refractory to cyclic nucleotide-dependent relaxation (5, 6).

Activation of cyclic nucleotide-dependent protein kinases leads not only to the active relaxation of precontracted bovine carotid artery smooth muscle but also to the inhibition of agonist-induced contractions. To investigate the relationship between myosin light chain phosphorylation and the cyclic nucleotide-dependent protein kinase signaling pathways in vascular smooth muscle, we examined the contractile activity, phosphorylation and the cyclic nucleotide-dependent protein kinase assay kit were purchased from Bio-Rad (Hercules, CA). The $^{[32P]}$orthophosphate and $^{13}$I-labeled protein A were purchased from ICN Biomedical Laboratories (Costa Mesa, CA).

Isometric force measurements. Bovine carotid arteries were dissected from fetal calves at a local abattoir (Shapiro's Meatpackers, Augusta, GA) and placed directly in cold HEPES buffer (140 mM NaCl, 4.7 mM KCl, 1.0 mM MgSO$_4$, 1.0 mM NaH$_2$PO$_4$, 1.5 mM CaCl$_2$, 10 mM glucose, and 10 mM HEPES, pH 7.4). The carotid vessels were dissected free from the adventitia and opened longitudinally. The endothelium was removed by rubbing the intima with a cotton-tipped applicator. Transverse strips 1.0 mm in width were cut, and each end was tied to a loop of 3-0 silk. The human umbilical arteries were obtained from normal full-term deliveries from the Department of Labor and Delivery with approval of the human assurance review board of the Medical College of Georgia and placed directly into cold HEPES buffer. The umbilical arteries were dissected free from the wharton jelly and cut transversely into rings 1.0 mm in width. The endothelium was denuded by inserting a small needle and thread into the lumen of the tissue ring. The rings were suspended using two loops of 3-0 silk. The tissue was bathed in a muscle bath containing a bicarbonate buffer (120 mM NaCl, 4.7 mM KCl, 1.0 mM MgSO$_4$, 1.0 mM NaH$_2$PO$_4$, 10 mM glucose, 1.5 mM CaCl$_2$, and 25 mM NaHCO$_3$, pH 7.4) equilibrated with 95% O$_2$-5% CO$_2$ at 37°C. All tissues were allowed to equilibrate for 4 h before experimental manipulation.

Changes in isometric force were registered by force transducers (Grass Instrument, Quincy, MA) and recorded on a chart recorder (Gould Instrument, Norcross, GA). Tissue rings were progressively stretched, and isometric force generated in response (110 mM KCl with equimolar replacement of NaCl in bicarbonate buffer) was monitored until optimal tension was produced (L$_{max}$). Agonists and inhibitors were added directly to the muscle bath. For Ca$^{2+}$-free conditions, Ca$^{2+}$ was omitted and the extracellular Ca$^{2+}$ chelator EGTA (4 mM) and intracellular Ca$^{2+}$ chelator BAPTA-AM (0.1 mM) were added to the buffer (36). After each experiment, tissues were blotted and their wet weight was measured. Force was converted to stress (10$^5$ N/m$^2$), calculated as force (g$\times$wet weight of the ring. Oxygen consumption rates were determined with a custom data collection system at a rate of 5 samples/s. The values are reported as the means ± SE of J O$_2$ values obtained during a 2-min sampling window. Stress measurements are reported as the force generated, normalized for ring cross-sectional area at a length for optimal force generation (37). The suprabasal oxygen consumption was calculated as the difference between prestimulated and experimental J O$_2$. Mean suprabasal oxygen consumption was calculated from the average of individual treatments.

Myosin light chain phosphorylation. Strips of bovine carotid artery or rings of human umbilical artery smooth muscle were equilibrated in a muscle bath as described in Isometric force measurements. and at the appropriate time points the muscles were snap-frozen with tongs cooled in liquid N$_2$. The tissues were then ground to a fine powder under liquid N$_2$. The powder was placed in 90% acetone, 10% TCA, and 10 mM dithiothreitol (DTT) and subsequently washed three times with 100% acetone and 100 mM DTT. The samples were lyophilized, and the pellet was resuspended in 9 M urea, 2% CHAPS, and 100 mM DTT. Twenty micrograms of protein were separated on glycerol-urea minigels (40%
95% O₂-5% CO₂ for 1 h. The tissues were then rinsed with saline (TBS)-milk (10 mM Tris, 150 mM NaCl, pH 7.4, 5% nonfat milk, and 0.5% Tween 20) and then incubated overnight with anti-MLC20 antibodies (1:4,000 in TBS-milk). After being washed (6 times for 5 min with TBS-Tween), the blots were incubated in 125I-protein A and the relative amounts of phosphorylated and nonphosphorylated MLC20 were quantitated with the PhosphorImager imaging system (Molecular Dynamics, Sunnyvale, CA).

The 125I radioactivity was quantitated with ImageQuant software (Molecular Dynamics). The amount of phosphorylation was measured from the volume of radioactivity in each band, nonphosphorylated and monophosphorylated. The moles of phosphorylated myosin light chain per mole of total myosin light chains were calculated.

Whole cell phosphorylation. Strips of bovine carotid artery smooth muscle and rings of human umbilical artery smooth muscle were equilibrated in bicarbonate buffer bubbled with 98% O₂-2% CO₂ for 1 h at 37°C. The tissues were then rinsed with 1 mM HEPES, pH 7.4, 140 mM NaCl, 4.7 mM KCl, 1.5 mM CaCl₂, 10 mM glucose, and 0.3 mM NaH₂PO₄ for 30 min. The tissues were then equilibrated in low-phosphate buffer containing 250 µCi/ml [32P]orthophosphate for 4 h.

After stimulation with the appropriate agonist, the muscles were snap-frozen in liquid N₂ and crushed with a mortar and pestle. The powder was resuspended in 90% acetone, 10% TCA, and 10 mM DTT and subsequently washed three times with 100% acetone and 10 mM DTT. The pellet was resuspended in 9 M urea, 2% CHAPS, and 100 mM DTT.

Two-dimensional gel electrophoresis. Two-dimensional gel electrophoresis (27) was performed using vertical slab isoelectric focusing gels with modifications (17). The conditions were optimized to emphasize the low-molecular-weight phosphorylated proteins (in the range of MLC20) by using an ampholine gradient of pH 4.5–7.5 and 12% SDS second-dimensional gel. Fifteen percent glycerol and five percent ampholines (5 parts 6:8, 3 parts 5:7, 2 parts 3:10) were added to 150 µg of protein in a final volume of 100 µl with 9 M urea, 2% CHAPS, and 100 mM DTT. The first dimensions were focused for 10,000 Vh, and then the gels were fixed in 12% TCA and stained with Coomassie brilliant blue G250, dried, and imaged using the PhosphorImager. The phosphorylated isoforms of HSP20 were analyzed for the amount of radioactivity above background using ImageQuant software. The area used for quantitating each isoform was the same. An area of the image that was devoid of signal was assigned the background value. Equal protein loading was confirmed by densitometric analysis of Coomassie-stained actin.

Data analysis. Values are reported as means ± SE. Statistical differences between groups were determined using ANOVA using SigmaStat software (Jandel Scientific, San Rafael, CA). A p value <0.05 was considered significant, and n refers to the number of animals examined for each experimental condition.

RESULTS

Contraction and protein phosphorylation of bovine carotid artery smooth muscle. Treatment of bovine carotid artery smooth muscle with serotonin (1 µM) led to a rapid, sustained contraction (Fig. 1A). This contraction was associated with a transient increase in myosin light chain phosphorylation (Fig. 1B) and no change in HSP20 phosphorylation (Fig. 1C). Treatment of carotid artery with IBMX (1 mM)-forskolin (10 µM) for 10 min before serotonin stimulation did not significantly lower resting stress (Fig. 2A) but decreased myosin light chain phosphorylation (Fig. 2B) and increased HSP20 phosphorylation (Figs. 2C and 3B). The subsequent addition of serotonin (1 µM) after IBMX + forskolin pretreatment did not increase stress (Fig. 2A). However, there was a transient increase in myosin light chain phosphorylation (Fig. 2B) and a sustained elevation of HSP20 phosphorylation (Figs. 2C and 3C). In addition, treatment of carotid artery with forskolin alone (10 µM, 10 min) followed by serotonin (1 µM, 60 min) stimulation completely inhibited the contractile response.
Inhibition of smooth muscle contraction by 3-isobutyl-1-methylxanthine (IBMX) + forskolin (FSK) with myosin light chain and HSP20 phosphorylation in bovine carotid artery. A: bovine carotid artery smooth muscles were equilibrated in a muscle bath and then treated with 1 mM IBMX + 10 µM FSK (IBMX + FSK) for 10 min (1st arrow) before stimulation with 1 µM 5-HT for 60 min (2nd arrow). B: some strips were snap-frozen, and MLC20 phosphorylation was determined. C: on separate strips the phosphorylation of HSP20 (isoform 3) was determined. *P < 0.05 compared with unstimulated controls (by ANOVA on ranks n = 4–10).

To determine whether myosin light chain kinase mediated the myosin light chain phosphorylation by serotonin stimulation after IBMX + forskolin pretreatment, we measured myosin light chain phosphorylation under Ca$^{2+}$-free conditions and in the presence of the myosin light chain kinase inhibitor ML-9. Serotonin-induced increases in myosin light chain phosphorylation in the presence of IBMX + forskolin were significantly inhibited by ML-9 (200 µM) and completely abolished under conditions in which free Ca$^{2+}$ was buffered using EGTA (4 mM) + BAPTA (0.1 mM) (Fig. 4).

Oxygen consumption in bovine carotid artery smooth muscle. To examine the energetic state of the carotid artery ring, oxygen consumption and stress were measured in rings stimulated with serotonin before and after incubation with IBMX + forskolin. Each ring was stimulated with serotonin for 60 min and then passively relaxed for 45 min. The rings were then treated with IBMX + forskolin for 15 min, followed by a second serotonin stimulation for 60 min. Serotonin stimulation produced a sustained increase in stress (Fig. 5A) associated with a transient increase in oxygen consumption (Fig. 5B). Stimulation with serotonin after pretreatment with IBMX + forskolin produced no increase in stress (Fig. 5A), but there was an increase in oxygen consumption (Fig. 5B). The transient increase in suprabasal oxygen consumption with serotonin stimulation was similar in the absence or presence of IBMX + forskolin (Fig. 5C).

Contraction and protein phosphorylation of human umbilical artery smooth muscle. Treatment of human umbilical artery smooth muscle with serotonin (1 µM) led to a rapid and sustained contraction (Fig. 6A). This contraction was associated with a transient increase in myosin light chain phosphorylation (Fig. 6B) and no change in HSP20 phosphorylation (Fig. 6C). Treatment of the muscle with IBMX + forskolin for 10 min resulted in decreased stress (Fig. 7A) with no change in myosin light chain phosphorylation (Fig. 7B) and no significant change in HSP20 phosphorylation (Figs. 7C.
and 3E). Pretreatment with IBMX + forskolin did not prevent a serotonin (1 µM)-induced contraction, but after 20 min the contraction began to drop toward prestimulus levels (Fig. 7A). The contraction was associated with transient increases in myosin light chain phosphorylation similar to those induced by serotonin stimulation alone (Fig. 7B). HSP20 phosphorylation slowly increased to levels significantly greater than basal (Figs. 7C and 3F). In addition, treatment of umbilical artery with forskolin alone (10 µM, 10 min), followed by serotonin (1 µM, 60 min) stimulation, did not inhibit the contractile response (0.035 ± 0.008 × 10^5 N/m² at 0 min; 0.033 ± 0.005 × 10^5 N/m² at 2 min; 0.070 ± 0.009 × 10^5 N/m² at 20 min; 0.068 ± 0.007 × 10^5 N/m² at 60 min) (Fig. 7A; only the 60-min time point is shown) and did not result in an increase in HSP20 phosphorylation (Fig. 7C).

**DISCUSSION**

Activation of cyclic nucleotide-dependent signaling pathways in bovine carotid artery smooth muscle before serotonin stimulation completely inhibits the contractile response. However, activation of these pathways does not prevent myosin light chain phosphorylation or oxygen consumption in response to serotonin stimulation. The inhibition of contraction was associated with increased phosphorylation of the small heat shock-related protein HSP20 (Fig. 2). These data suggest that activation of cyclic nucleotide-dependent signaling pathways leads to increases in the phosphorylation of HSP20 but not to the inhibition of myosin light chain phosphorylation.

Increases in the phosphorylation of the myosin light chains can occur through activation of specific kinases, such as the Ca^2+^-dependent myosin light chain kinase or the recently described Rho-kinase, or through inactivation of the myosin light chain phosphatase (22, 35). We tested whether the increases in the phosphorylation of the myosin light chains were caused by the action of the Ca^2+^-dependent myosin light chain kinase in bovine carotid artery smooth muscle. The myosin light chain phosphorylation was inhibited by Ca^2+^-free conditions and by ML-9, a myosin light chain kinase inhibitor (Fig. 4). This suggests that the myosin light chain phosphorylation seen during serotonin stimulation after IBMX + forskolin pretreatment is caused by the Ca^2+^-dependent activation of myosin light chain kinase and not by the actions of other kinase pathways.

Several investigators have described a dissociation between myosin light chain phosphorylation and the production of force in various smooth muscles (for review, see Ref. 2). Activation of cyclic nucleotide-dependent pathways leads to active relaxation of the smooth muscle without inhibition of myosin light chain phosphorylation (1, 24). However, nearly all of these studies were performed by first precontracting the muscle with a contractile agonist and then activating the cyclic nucleotide pathway. Our results demonstrate an uncoupling of initial force development from myosin light chain phosphorylation when the artery is pretreated with activators of cyclic nucleotide-dependent signaling pathways and then stimulated to contract with serotonin. In addition, these data reveal the uncoupling of force from myosin light chain phosphorylation in a setting in which there are increases in myosin light chain phosphorylation that are sufficiently large enough to activate cross bridges and initiate contraction.

There are two potential mechanisms to explain the cyclic nucleotide-dependent dissociation between force development and myosin light chain phosphorylation/oxygen consumption. The first is that activation of
these pathways may prevent phosphorylated myosin from interacting with actin, resulting in abnormally functioning cross bridges. Our oxygen consumption and myosin light chain phosphorylation data would suggest such a mechanism is unlikely. In vitro biochemical measurements of ATPase rates of smooth muscle myosin and heavy meromyosin (HMM) have shown a dependence on the myosin phosphorylation state. The intrinsic ATPase rate for phosphate release of unphosphorylated HMM was 0.002 s$^{-1}$ in the absence of actin (13). Phosphorylation of HMM resulted in a fourfold increase in the ATPase rate of phosphate release in the absence of actin (31, 32). In contrast, there were nearly 1,000-fold increases in ATPase activity of phosphorylated HMM in the presence of actin (13, 32). The presence of nearly identical rates of oxygen consumption and myosin light chain phosphorylation patterns in the carotid artery suggests that cross bridges have been activated and have normal interaction with the actin filaments. Because myosin light chain phosphorylation is necessary for cross-bridge activation and actomyosin ATPase activity (34), any impaired activation would result in a lower energy

Fig. 5. Oxygen consumption in bovine carotid artery increases with 5-HT stimulation to a similar extent with or without IBMX + FSK pretreatment. Bovine carotid artery smooth muscle rings were mounted in a sealed muscle bath chamber designed to monitor force and oxygen tension concurrently. Muscle was treated with 1 mM IBMX + 10 µM FSK for 15 min, followed by 1 µM 5-HT stimulation for 60 min. Stress (A), rate of oxygen consumption ($J_{O2}$; B), and suprabasal $J_{O2}$ (C) are shown with 5-HT stimulation with and without IBMX + FSK pretreatment. $^*P < 0.05$ compared with time 0 of control (by ANOVA on ranks $n = 6$–8).

Fig. 6. Contraction, myosin light chain phosphorylation, and HSP20 phosphorylation after 5-HT stimulation in human umbilical artery. A: human umbilical artery smooth muscles were equilibrated in a muscle bath and stimulated with 1 µM 5-HT (arrow). B: some strips were snap-frozen, and MLC$_{20}$ phosphorylation was determined. C: on separate strips the phosphorylation of HSP20 (isoform 3) was determined. $^*P < 0.05$ compared with unstimulated controls (by ANOVA on ranks $n = 5$–12).
demand and be indirectly reflected in a lower rate of oxygen consumption. A second possibility is a dissociation between the contractile apparatus and specific focal contacts, such as dense bodies and dense plaques, which provide a framework for the attachment of contractile structures to the cytoskeleton (20, 29). This could result in normal cross-bridge phosphorylation and interactions, resulting in a similar energetic demand. The nearly identical increase in the rate of oxygen consumption would suggest the uncoupling of the normally activated contractile apparatus from focal contacts as a possible mechanism for relaxation in the carotid artery.

It is also possible that other ATPases are activated during the IBMX + forskolin treatment. However, other active ATPases would require rates approaching that of the ATPase of the phosphorylated myosin filament to be able to match the oxygen consumption rates. Inhibition of activation of contraction in swine carotid smooth muscle using ML-9 and wortmannin resulted in an inhibition of oxygen consumption as well as myosin light chain phosphorylation and force production (38, 39). This suggests that the major energetic pathway for smooth muscle contraction involves the phosphorylation of the cross bridge- and actin-activated ATPase activity.

The umbilical artery has been reported to be uniquely refractory to cyclic nucleotide-dependent vasorelaxation (5). Maximal activation of cyclic nucleotide-dependent signaling pathways with the phosphodiesterase inhibitor IBMX and the adenylate cyclase activator forskolin did not prevent increases in stress caused by serotonin stimulation. However, the rate of stress production and the maximal stress produced were decreased. In addition, the contractions were not sustained, and the decrease in stress was associated with a progressive, but slow, increase in the phosphorylation of HSP20. With the use of forskolin alone, the subsequent contractile response to serotonin was sustained and there was no significant increase in the phosphorylation of HSP20. These data suggest that the extent of HSP20 phosphorylation correlates with the relaxation of serotonin-induced contraction in the umbilical smooth muscle.

The umbilical smooth muscle has higher resting tone than the carotid smooth muscle. This has been attributed to increased intracellular Ca\(^{2+}\) in umbilical smooth muscle. Decreasing extracellular Ca\(^{2+}\) or pretreatment with nifedipine leads to greater relaxation in the presence of nitrovasodilators (43). This suggests that there may be multiple, interrelated pathways that contribute to the cyclic nucleotide-dependent relaxation of vascular smooth muscles.

The association between increases in HSP20 phosphorylation and the inhibition of contraction suggests that HSP20 may be important in modulating this process. We have recently identified the site on the HSP20 molecule that is phosphorylated during cyclic nucleotide-dependent relaxation as serine-16 (3). The introduction of phosphopeptide analogs of HSP20 into permeabilized bovine carotid artery smooth muscles inhibited serotonin-induced contractions (3). Finally, HSP20 is an actin-associated protein, and the association between HSP20 and actin in vitro is dependent on the phosphorylation state of HSP20 (7). Because the small heat shock proteins are molecular chaperones, it is possible that HSP20 is directly modulating the interaction of specific cytoskeletal and/or contractile proteins, possibly at the dense bodies or dense plaques.

In conclusion, the phosphorylation of HSP20 correlates with the inhibition of agonist-induced contraction. In the carotid artery, HSP20 phosphorylation is maximal after 10 min, resulting in complete inhibition of serotonin-induced contractions, whereas in the umbilical artery, HSP20 phosphorylation occurs over an extended time period, leading to a progressive decrease in...
force. However, activation of cyclic nucleotide-dependent signaling pathways does not inhibit myosin light chain phosphorylation or oxygen consumption. Taken together, these data suggest that the mechanisms of cyclic nucleotide-dependent vasorelaxation are associated with HSP20 phosphorylation and are independent of the Ca$^{2+}$-dependent myosin light chain phosphorylation pathway.

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