Stretch Induces Mitogen-Activated Protein Kinase Activation and Myogenic Tone Through 2 Distinct Pathways

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Abstract—The aim of this study was to evaluate the involvement of the mitogen-activated protein kinase (ERK1/2) pathway in response to stretch in a blood vessel developing myogenic tone on stretch. Indeed, in resistance arteries and veins, the main effect of pressure is to induce a maintained vasoconstrictor (myogenic) tone. Isolated segments of rabbit facial vein were mounted in organ baths and submitted to isometric stretch. In this experimental model, myogenic tone was absent when the bath temperature was 33°C. ERK1/2 activity was determined in each isolated segment by an in-gel kinase assay. Wall tension and ERK1/2 activity were measured in the same samples in the presence (39°C) or in the absence of myogenic tone (33°C). At 39°C, a 5-mN wall tension induced myogenic tone (5.7 ± 1.8 mN) and an increase in ERK1/2 activity (282 ± 52% versus unstretched vessels, P < 0.05). At 33°C, in the absence of myogenic tone, ERK1/2 activity was similarly increased by stretch (254 ± 35% versus unstretched vessels). The calcium-dependent and -independent protein kinase C (PKC) blocker Ro-31-8220 (5 × 10⁻⁷ mol/L), but not the calcium-dependent PKC blocker Go-6976 (10⁻⁶ mol/L), inhibited myogenic tone. However, ERK1/2 activity was not affected by either PKC blocker. Genistein (10⁻⁷ mol/L), a general tyrosine kinase inhibitor, but not herbimycin A (5 × 10⁻⁷ mol/L), a cSrc-family tyrosine kinase inhibitor, suppressed stretch-induced ERK1/2 activation (P < 0.05) without affecting myogenic tone. Nifedipine (10⁻⁶ mol/L), a voltage-dependent calcium entry inhibitor, and ryanodine (10⁻⁶ mol/L), which depletes calcium stores, both inhibited ERK1/2 activity (113 ± 12% and 121 ± 7%, respectively; P < 0.05) without affecting myogenic tone. The mitogen-activated protein kinase kinase inhibitor PD 98059 (5 × 10⁻⁶ mol/L) also inhibited ERK1/2 activation without affecting myogenic tone. The present results suggest that stretching the rabbit facial vein induced 2 distinct pathways, one leading to myogenic tone (via a non–calcium-dependent PKC activation) and one leading to ERK1/2 activation through a calcium-dependent pathway involving tyrosine kinase.

Key Words: protein kinase C • stretch • extracellular signal–related kinase • mitogen-activated protein kinase • rabbits

Pressure in resistance arteries and veins is responsible for a sustained contraction, which is mainly of myogenic origin. Myogenic tone is a major phenomenon in the regulation of regional blood flow. Although resistance vessels represent the majority of the arterial tree, their sizes limit the possibility to perform biochemical measurements needed to identify the signaling pathways involved in the response to pressure. In these vessels, myogenic tone is decreased by protein kinase C (PKC)² and phospholipase C³ inhibitors. In isolated cells, stretch leads to the opening of stretch-activated cationic channels⁴ and to the activation of phosphoinositide turnover,⁵ PKC,⁶ and calcium/calmodulin-dependent kinases.⁷ In addition, although calcium entry is required for the development of myogenic tone,⁸ the amount of calcium needed is much smaller than necessary for agonist-induced tone.⁹ Activation by stretch of the different transduction pathways leads to immediate cellular responses, such as secretion of bioactive substances,¹⁰ activation of smooth muscle cell contraction,¹¹ and cytoskeletal rearrangement.¹² Finally, a recent study has shown that pressure-induced (myogenic) tone in rat resistance cerebral arteries was sensitive to the tyrosine kinase inhibitor herbimycin A.¹³ The mitogen-activated protein (MAP) kinase pathway plays an important role in the signal transduction in vascular smooth muscle and endothelial cells. Indeed, extracellular signal–related kinases (ERKs) 1 and 2 are involved in the mechanotransduction of flow in endothelial cells¹⁴ and of pressure in the vascular smooth muscle.¹⁵,¹⁶ We have previously reported that in a model of aortic organ culture, ERK1/2 was activated in vascular smooth muscle cells in response to elevated intraluminal pressure.¹⁶ However, there is no information concerning the activation of ERK1/2 in blood vessels developing myogenic tone. The rabbit facial vein (RFV) develops a myogenic tone similar to that observed in resistance arteries.³,¹⁷ Furthermore, myogenic tone is abolished in the RFV that is exposed to low intraluminal pressure.¹⁸

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2878
temperature (33°C). Therefore, we used this experimental model to test the effect of vascular wall stretch on ERK1/2 activity in the presence (39°C) or the absence (33°C) of myogenic tone and to determine whether ERK1/2 activation is involved or affected buy the development of myogenic tone.11-18

**Methods**

**Rabbit Facial Vein**

Buccal segments of RFV were isolated as previously described.11 Ring segments (3 mm long) of RFV were mounted between parallel stainless steel wires in 5-mL organ baths. One wire was attached to a fixed support; the other was connected to a moveable holder supporting a tension transducer, so that isotonic force could be recorded. Data were collected by a Biopac data acquisition system (Biopac MP 100). The vein segments were maintained at 39°C in a physiological salt solution with the following composition (in mmol/L): NaCl 135.0, NaHCO3 15.0, KCl 4.6, CaCl2 1.5, MgSO4 1.2, glucose 11.0, and HEPES 10.0, pH 7.4. P02 was maintained at a value of 160 mm Hg; P CO2, at a value of 37 mm Hg. After a 30-minute equilibration period, the segments were subjected to a 5.0-nm force (stretch), which is optimal for the development of myogenic tone.11 The procedure followed in the care and euthanasia of the study animals was in accordance with the European Community standards on the care and use of laboratory animals (authorization No. 00577).

**Experimental Protocol**

ERK1/2 activity was determined as previously described,15 and RFV segments were submitted to one of the following protocols. Each sample of vein was divided in 2 segments. One of the 2 segments was used as a control vessel,16 and the other was mounted in the myograph for force measurement. ERK1/2 activity was measured in both segments. The activity of each sample that had been mounted in a myograph and submitted to an experimental protocol was calculated as a percentage of the activity found in the corresponding control unstretched segment.16

At the end of each experimental protocol, the vein segment was rapidly removed from the organ bath, frozen in liquid nitrogen, and subsequently stored at −80°C until determination of ERK1/2 activity.

In a first series of experiments, RFV segments were stretched to an optimal tension (5 mN) and allowed to stabilize for 30 minutes in the absence (33°C, n=8) or in the presence (39°C, n=11) of myogenic tone.

In a second series of experiments, RFV segments were stretched at 39°C. Veins were then exposed during 30 minutes to temperature from 39°C to 33°C totally suppressed myogenic tone.11 Decreasing the temperature from 39°C to 33°C totally suppressed myogenic tone. There was no difference between 33°C and 0-calcium solution at 33°C containing sodium nitroprusside (10-4 mol/L) and EGTA (10-4 mol/L). At 39°C in the presence of PD Bu (10-4 mol/L), myogenic tone increased significantly (10.7±1.8 mN), whereas the PKC inhibitor Ro-31-8220 (5×10-7 mol/L) totally inhibited myogenic tone.

ERK1/2 activity was determined in RFV segments submitted to stretch for 30 minutes (Figure 2). In vein segments
The role of tyrosine kinases in stretch-induced ERK1/2 activity and myogenic tone was assessed by using 2 inhibitors (Figure 5). Genistein (10^{-7} mol/L), a broad tyrosine kinase inhibitor, abolished ERK1/2 activity (121.8±50.2%, n=9 stretched vessels), whereas herbimycin A (5×10^{-7} mol/L), a c-src family tyrosine kinase inhibitor, did not affect ERK1/2 activity (276±12.3%, n=7 stretched vessels). Neither genistein nor herbimycin A significantly affected myogenic tone. The specific MAP kinase kinase inhibitor, PD 98059 (5×10^{-6} mol/L), significantly decreased ERK1/2 activity at 39°C (33.2±8.4% decrease in stretched vessels [n=6] versus unstretched vessels) without affecting myogenic tone. Similarly, PD 98059 decreased ERK1/2 activity at 33°C (29.4±4.6% decrease in stretched vessels [n=6] versus unstretched vessels).

Both the voltage-dependent calcium channel inhibitor nifedipine (10^{-6} mol/L) and ryanodine (10^{-6} mol/L), which depletes calcium stores, inhibited ERK1/2 activity (Figure 6) (113±12.4%, n=6, and 121±7.4%, n=6, respectively) without affecting myogenic tone (Figure 6). On the other hand, in vessels bathed in a 0-calcium physiological salt solution+EGTA (2 mmol/L), both myogenic tone and ERK1/2 activity were abolished.
Discussion

The main finding of the present study is that acute stretch in an RFV triggers 2 independent pathways, one leading to the development of myogenic tone and one leading to the activation of ERK1/2.

Although myogenic tone is mainly studied in resistance arteries, it also occurs in veins. As pointed out by Monos, only recently have veins been considered “an active component of the cardiovascular system.” In veins, myogenic tone is an active regulator of systemic venous capacity. Myogenic tone in the RFV is very similar to that in resistance arteries, but it is temperature sensitive. This unique feature was used to measure ERK1/2 activation on stimulation by stretch in the presence or in the absence of myogenic tone.

The ERK1/2 pathway is involved in a rapid transduction of growth signals and mechanical strain into regulation of gene expression and protein synthesis. Cyclic stretch in cardiac myocytes stimulates several kinases, including tyrosine kinases, ERK1/2, and PKC. The ERK1/2 pathway is involved in the vascular smooth cell response to contractile and proliferative stimuli. In addition, mechanical stretch leads to the activation of the ERK1/2 pathway in vivo.

The implication of calcium in myogenic tone has been previously shown in arteries and veins. The degree of involvement of voltage-activated channels is dependent on the type of vessel studied. Voltage-activated channels play a major role in cerebral arteries, whereas they are only partly involved in coronary arteries and do not play a role in the RFV (present study). A role for PKC activation in myogenic tone has been previously suggested in studies in which myogenic tone was inhibited by pharmacological agents in arteries and veins. Nevertheless, in these studies, the selectivity of the PKC inhibitors was rather weak. Using recent and more selective PKC inhibitors, we could show that calcium-independent PKC(s) are involved in myogenic tone in the RFV. This observation is compatible with previous studies showing that myogenic tone in this vessel depends on a low increase in intracellular calcium, suggesting that myogenic tone might be related to a PKC-dependent sensitization of the contractile apparatus to calcium in the RFV and in resistance arteries. We have previously shown that myogenic tone in the RFV is related to a much lower ratio of calcium influx to force than other forms of tone, such as agonist- or KCl-induced tone. Nevertheless, further investi-
gation might be needed to obtain more direct evidence that a sensitization of the contractile apparatus may occur when myogenic tone develops. However, myogenic tone was not sensitive to tyrosine kinase or MAP kinase kinase blockade, suggesting that its development does not require prior activation of the ERK1/2 activation pathway. Both in the presence or in the absence of myogenic tone, ERK1/2 activation by stretch was inhibited by the MAP kinase kinase inhibitor PD 98059. In addition, ERK1/2 activation was sensitive to genistein but not to herbimycin A, suggesting the absence of involvement of c-src in the process. This is at variance with previous studies in the rabbit aorta showing that pressure-induced stretch activates ERK1/2 through a PKC-independent and a c-src–dependent signaling pathway. This difference may be explained by a difference in vessel type (artery versus vein) or vessel caliber. ERK1/2 activation was also dependent on a calcium entry through voltage-operated channels and on a calcium release from the sarcoplasmic reticulum. PKC activation was not necessary for the activation of ERK1/2 by stretch. The kinetics of activation of ERK1/2 was similar in the 2 types of vessels. In swine carotid arteries, stretch-induced ERK1/2 activation is only partly dependent on calcium.

The level of ERK1/2 activation was similar at 33°C and 39°C, and the inactivation of ERK1/2 by PD 98059, a specific MAP kinase inhibitor, was total at both temperatures. Thus, we can exclude a specific effect of temperature on ERK1/2 activation.

The dissociation between force development on stretch (myogenic tone) and stretch-induced ERK1/2 activation is in agreement with recent studies that have reported a similar dissociation between wall force and ERK1/2 activation in swine carotid arteries stimulated by histamine and phorbol ester and in the rat aorta in response to angiotensin II. Thus, 2 distinct pathways were activated by stretch. One led to myogenic tone through a PKC-dependent pathway, and one led to ERK1/2 activation through a calcium-dependent and PKC-independent pathway.

References

4. Langton PD. Calcium channel currents recorded from isolated myocytes of rat basilar artery are stretch sensitive. *J Physiol (Lond)*. 1993;471:1–11.


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