Different roles of PKC and MAP kinases in arteriolar constrictions to pressure and agonists

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Massett, Michael P., Zoltan Ungvari, Anna Csiszar, Gabor Kaley, and Akos Koller. Different roles of PKC and MAP kinases in arteriolar constrictions to pressure and agonists. Am J Physiol Heart Circ Physiol 283: H2282–H2287, 2002; 10.1152/ajpheart.00544.2002.—Protein kinase C (PKC) and mitogen-activated protein (MAP) kinases have been implicated in the modulation of agonist-induced constrictions of large vessels. However, their role in pressure- and agonist-induced constrictions of skeletal muscle arterioles, which have a major role in regulating peripheral resistance, is not clearly elucidated. Thus constrictions of isolated rat gracilis muscle arterioles (∼80 μm in diameter) to increases in intraluminal pressure and to norepinephrine (NE) or angiotensin II (ANG II) were assessed in the absence or presence of chelerythrine, PD-98058, and SB-203580 (inhibitors of PKC, p42/44 and p38 MAP kinase pathways, respectively). Arteriolar constriction to NE and ANG II were significantly reduced by chelerythrine (by ∼90%) and unaffected by SB-203580, whereas PD-98058 decreased only ANG II-induced constrictions (by ∼60%). Pressure-induced increases in wall tension (from 0.1 to 0.7 N/m) resulted in significant arteriolar constrictions (50% maximum) that were abolished by chelerythrine without altering smooth muscle intracellular Ca2+ concentration ([Ca2+]i) (fura 2 microfluorimetry). PD-98058 and SB-203580 significantly decreased the magnitude of myogenic tone (by 20% and 60%, respectively) and reduced the sensitivity of the myogenic mechanism to wall tension, causing a significant rightward shift in the wall tension-myogenic tone relationship without affecting smooth muscle [Ca2+]i. MAP kinases were demonstrated with Western blotting. Thus in skeletal muscle arterioles 1) PKC is involved in both myogenic and agonist-induced constrictions, 2) PD-98058-sensitive p42/44 MAP kinases modulate both wall tension-dependent and ANG II-induced constrictions, whereas 3) a SB-203580-sensitive p38 MAP kinase pathway seems to be specifically involved in the mechanotransduction of wall tension.

microvessels; myogenic constriction; PD-98059; SB-203580; smooth muscle; protein kinase C; mitogen-activated protein

PERIPHERAL RESISTANCE depends on the tone of small arteries and arterioles, which is regulated by neural, humoral factors [e.g., angiotensin II (ANG), norepinephrine (NE)], and local mechanisms intrinsic to the vessel wall (17, 18). Among the intrinsic mechanisms, the pressure-sensitive arteriolar myogenic response plays an important role in the local regulation of resistance, hence tissue blood flow (6, 8, 22, 33, 37, 38). It is thought that by reducing vessel diameter the pressure-induced response represents a negative feedback mechanism that helps to maintain wall tension according to the law of Laplace and Frank (17, 18). However, the pathways in arteriolar smooth muscle that contribute to the mechanotransduction of pressure-wall tension have not been clearly elucidated (8, 15, 30).

It has been shown that increases in pressure-wall tension are associated with increases in smooth muscle intracellular Ca2+ concentration ([Ca2+]i) due to the influx of extracellular Ca2+ and constriction (42, 50). Recent studies also raised the possibility that pressure-wall tension activates Ca2+ independent pathways and/or pathways that increase the sensitivity of the contractile machinery to Ca2+ (8, 15, 27, 30, 49). These cascades were shown to involve PKC (1, 11, 49); however, the roles of mitogen-activated protein (MAP) kinases, which may also affect smooth muscle contractility (24), in arteriolar myogenic constriction have not been fully explored.

From the 12 identified MAP kinases, the p42/44 (ERK1/2) and p38 MAP kinase pathways were proposed as likely modulators of vascular smooth muscle contractions (8, 24, 28, 29, 46, 48). Studies on large vessels and isolated smooth muscle cells suggested that activation of MAP kinases contribute to vascular smooth muscle contraction induced by agonists (4), such as NE and ANG II (29, 39). Interestingly, in conduit vessels that do not develop spontaneous tone in response to increases in intraluminal pressure, MAP kinases were also shown to be phosphorylated and activated in vitro by mechanical stretch (2, 26, 32, 36), whereas pharmacological inhibition of p42/44 MAP kinase activation was reported to abolish the tone developed by middle cerebral arteries to 80 mmHg intraluminal pressure (24).

Because of the aforementioned findings, we hypothesized that the roles of PKC, p42/44, and/or p38 MAP kinases are different in agonist- and pressure-induced

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responses of skeletal muscle arterioles and aimed to elucidate whether specific relationships exist among these pathways. Thus changes in diameter of isolated rat gracilis muscle arterioles were obtained as a function of changes in intraluminal pressure-wall tension and in response to NE and ANG II before and after inhibition of the PKC, p42/44 MAP kinase, or p38 MAP kinase pathways.

METHODS

Isolation of arterioles: videomicroscopy. Skeletal muscle arterioles were prepared as previously described in detail (37, 43). Briefly, male Wistar rats (387 ± 3 g) were housed in an American Association for Accreditation of Laboratory Animal Care-accredited animal care facility at the New York Medical College, and all protocols were approved by the Institutional Animal Care and Use Committee. Animals were anesthetized with pentobarbital sodium (50 mg/kg ip), and the gracilis muscle was removed and placed in a dissecting dish containing cold (4°C) physiological saline solution (PSS) containing (in mmol/l): 118.3 NaCl, 24 NaHCO3, 4.7 KCl, 1.2 MgSO4, 1.2 KH2PO4, 1.9 CaCl2, and 11.1 glucose (pH 7.4). Arterioles were isolated and mounted onto two glass micropipettes in a vessel chamber (15 ml) and slowly pressurized to 80 mmHg by using a pressure-servo syringe respiration system (Living Systems Instruments, www.livingsys.com). PSS flow through the system was set at 40 ml/min. Inner arteriolar diameter was measured with a video micrometer system and continuously recorded using a computerized data acquisition system (Biopac MP100; Goleta, CA). All vessels were allowed to stabilize for 60 min in oxygenated (10% O2-5% CO2-85% N2) PSS warmed to 37°C.

Experimental protocols. After an equilibration period, changes in arteriolar diameter in response to ANG II (10−9, 10−6 mol/l) and NE (10−9,10−6 mol/l) were obtained at an intraluminal pressure of 80 mmHg before and after incubation with the benzophenanthridine alkaloid chelerythrine, an inhibitor of the PKC, p42/44 MAP kinase, or p38 MAP kinase (7). Concentration-response curves for NE were obtained (at 80 mmHg intraluminal pressure). Then arterioles were incubated with chelerythrine, PD-98059, or SB-203580. After an equilibration period, changes in arteriolar diameter in response to ANG II (10−6 mol/l), PD-98059 (10−5 mol/l), SB-203580 (5 μmol/l), or nimodipine (10−6 mol/l, an L-type Ca2+ channel antagonist) were obtained (at 80 mmHg intraluminal pressure). Drugs and solutions. PD-98059, SB-203580, chelerythrine, and fura 2 AM were initially dissolved in DMSO (0.01% of total volume) and then diluted with PSS. Final concentrations and incubation time for PD-98059, SB-203580, (7, 24, 26, 28, 29, 46), and chelerythrine (1, 21) were based on previously published data. All other drugs were dissolved in distilled water. The vehicle had no effect on arteriolar responses. PD-98059 was purchased from Cal-Biochem (San Diego, CA); all other chemicals were purchased from Sigma Chemical.

Data analysis. The slope of the pressure-diameter relationship between 40 and 100 mmHg for each curve was determined by linear regression analysis. Myogenic tone was calculated by dividing the active diameter by the passive diameter (expressed as percent) at each pressure step. Circumferential wall tension was calculated as: Tw = Pi × ri, where Tw is the circumferential wall tension, Pi is intraluminal pressure, and ri is the vessel radius (22, 50). The 50% effective value of Tw (T50) was calculated from the linear part of the wall tension-myogenic tone curves. The amplitudes of agonist- and pressure-induced constrictions were normalized (%) to the passive diameter at 80 mmHg. Constrictor responses before and after treatment were compared by using ANOVA for repeated measures followed by a modified Student’s t-test with the Bonferroni correction for multiple comparisons. Pre- and posttreatment comparisons were made by using paired Student’s t-tests when appropriate. Statistical significance was set at P < 0.05. Data are expressed as means ± SE.

RESULTS

Arteriolar constrictions to NE and ANG II. NE elicited concentration-dependent constrictions of isolated rat gracilis muscle arterioles that were abolished by 10 μmol/l chelerythrine (Fig. 1A). Inhibition of the p42/44
MAP kinase pathway with 10 μmol/l PD-98059 or inhibition of the p38 MAP kinase with 5 μmol/l SB-203580 did not significantly alter the maximal NE-induced constriction (Fig. 1A).

ANG II constricted arterioles in a concentration-dependent manner, a response that was abolished by chelerythrine (Fig. 1B). Arteriolar constrictions to ANG II were significantly inhibited by PD-98059 (Fig. 1B). In contrast, SB-203580 had no effect on arteriolar constrictions to ANG II (Fig. 1B).

Pressure-induced arteriolar constriction. In the absence of Ca2+ in the bath solution, step increases in pressure elicited continuous increases in arteriolar diameter (Fig. 2A; passive diameter at 80 mmHg: 132 ± 4 μm). In the presence of Ca2+, arterioles developed active myogenic tone in response to step increases in intraluminal pressure (20–140 mmHg) without the use of any vasoactive agent (Fig. 2A). Initially, the diameter of the vessels increased from ~85 μm to ~100 μm in response to an increase in intraluminal pressure from 20 to 40 mmHg. Beyond this point, further increases in pressure resulted in constrictions of arterioles. Inhibition of both PKC (10 μmol/l chelerythrine) and p38 MAP kinases (5 μmol/l SB-203580, Fig. 2A) altered the shape of the pressure-diameter curve as indicated by the slopes of the regression line fitted to the pressure-diameter relationship between 40 and 80 mmHg (control: −0.76 ± 0.18, chelerythrine: 0.02 ± 0.05, SB-203580: −0.08 ± 0.15; P < 0.05). Inhibition of the p42/44 MAP kinase pathway with 10 μmol/l PD-98059 also resulted in significantly increased arteriolar diameter at each pressure step (Fig. 2A). However, in the presence of PD-98059 the shape of the myogenic curve was similar to control (slope: −0.73 ± 0.06, not significant).

Wall tension-myogenic tone relationship. To further analyze the effect of inhibition of the PKC and MAP kinase pathways on the pressure-sensitive myogenic machinery of arterioles, from our data we calculated myogenic tone (percentage of corresponding passive diameter) and plotted these values against the calculated circumferential wall tension (Fig. 2B). In control conditions the wall tension-myogenic tone relationship was linear in the range of 0.2–0.5 N/m (slope: 1.3). Inhibition of the p42/44 MAP kinase pathway with PD-98059 resulted in a parallel rightward shift in the wall tension-myogenic tone relationship, indicating decreased tension sensitivity of the myogenic mechanism, without any significant change in the slope (1.3). In contrast, inhibition of both the p38 MAP kinase pathway with SB-203580 and PKC with chelerythrine resulted in a significant decrease in the slope of the wall tension-myogenic tone relationship (slope: 0.3 and 0.1, respectively) and an increased T50 (Fig. 2C).

Pressure-induced activation of PKCa, p42/44, and p38 MAP kinases. In arterioles submitted to pressure (80 mmHg), phosphorylation of PKCa (by ~170%) compared with nonpressurized vessels (Fig. 2D). Pressure also increased phosphorylation of p42/44 MAP kinase (by −114%), which was prevented by the same concentration of PD-98059 that was used in the functional studies (Fig. 2D). Phosphorylation of p38 MAP kinase was also increased by pressure (by −42%, Fig. 2D).

Measurement of smooth muscle [Ca2+]. Incubation of arterioles (pressurized to 80 mmHg) with chelerythrine (10 μmol/l), PD-98059 (10 μmol/l), or SB-203580 (5 μmol/l) did not significantly alter smooth muscle [Ca2+]; (Fig. 2D). In contrast, administration of the Ca2+ channel inhibitor nimodipine elicited a significant decrease in smooth muscle [Ca2+]; (Fig. 2D).

**DISCUSSION**

The new findings of the present study are that in skeletal muscle arterioles 1) constrictions to ANG II are inhibited by the PKC inhibitor chelerythrine (10 μmol/l) and PD-98059 (10 μmol/l), an inhibitor of the p42/44 MAP kinase pathway, but not by SB-203580 (5 μmol/l), an inhibitor of the p38 MAP kinase pathway; 2) constrictions to NE are inhibited by chelerythrine, whereas they are not affected by PD-98059 and SB-203580; and 3) myogenic constriction is inhibited completely by chelerythrine and partially by PD-98059 and SB-203580.

Mechanical forces, such as pressure-wall tension and pharmacological stimuli, may activate multiple signaling pathways in arteriolar smooth muscle that determine microvascular tone development. Previous stud-
ies demonstrated that in addition to increases in [Ca^{2+}]_i, activation of various kinase cascades regulating Ca^{2+} sensitivity of the contractile apparatus may modulate the level of arteriolar constriction (8, 15, 30). In the present study, we conducted experiments on isolated skeletal muscle arterioles to compare the role of the PKC, p42/p44 MAP kinase, and p38 MAP kinase pathways in the modulation of constrictions to agonists and pressure-wall tension by using pharmacological inhibitors. We found that ANG II- and NE-induced constrictions of skeletal muscle arterioles involve activation of PKC (Fig. 1A), extending previous findings on vessels from other vascular beds (25). Moreover, it is likely that p42/p44 MAP kinases are also involved in the signal transduction of ANG II, because ANG II-induced arteriolar constrictions were significantly inhibited by PD-98059 (Fig. 1A) adding previous findings in vascular smooth muscle cells (39, 40). Also, PD-98059 was shown to significantly decrease ANG II responses of rat mesenteric and human subcutaneous arterioles (28, 39, 40). Moreover, PD-98059 was shown to lower blood pressure in ANG II-induced hypertension in rats (31). It has been noted, however, that there are important tissue- and/or stimulus-specific differences in the effects of various agonists on the p42/44 MAP kinase pathway. For example, Watts (46) reported that PD-98059 reduced serotonin- but not ANG II-induced contraction of rat aortic rings. In contrast, at the level of arterioles, p42/44 MAP kinases, unlike PKC, play only a minor role, if any, in NE-induced vascular responses, because in the present study arteriolar constrictions to NE were essentially unaffected by PD-98059 (Fig. 1B). Similarly, PD-98059, while preventing α-adrenergic receptor-dependent activation of p42/44 MAP kinase, did not affect NE-induced contractions of rat aortic rings under control conditions, but it did so in the absence of extracellular Ca^{2+} (9). Arteriolar responses to NE were also insensitive to inhibition of p38 MAP kinase (Fig. 1B). A recent study reported that in the rat aorta, ANG II-induced contractions can be blocked by SB-203580 (29). In contrast, in the present study in skeletal muscle arterioles, SB-203580 did not alter ANG II-induced contractions (Fig. 1A). Thus it seems that the p38 MAP kinase pathway, unlike p42/44 MAP kinases, does not play a substantial role in agonist-induced arteriolar constrictions.

Myogenic constriction of skeletal muscle arterioles depends on both the influx of extracellular Ca^{2+} and the activity of the PKC pathway (Fig. 2, A and D), as shown in the present and previous studies (1, 10, 14,
Because PKC inhibitors abolish both agonist- and pressure-induced constrictions (1), it is likely that PKC represents a common pathway leading to arteriolar constriction shared by several stimuli. In contrast, PD-98059 elicited a significant rightward shift in the wall tension-myogenic tone relationship without altering its slope (Fig. 2, B and C), suggesting that the p42/p44 MAP kinase pathway modulates the sensitivity of the myogenic mechanism to wall tension, although it may not be essential for the development of pressure-induced constriction (36). The findings that the p38 MAPK inhibitor SB-203580 significantly attenuated both the maximal myogenic tone and the sensitivity of the myogenic mechanism to wall tension (Fig. 2, A–C) suggest that in gracilis muscle arterioles activation of the p38 MAP kinase pathway is involved in the mechanotransduction of wall tension. From previous studies, it can be hypothesized that changes in wall tension are sensed by extracellular matrix-coupled integrins that activate tyrosine phosphorylation events (8), which lead to activation of MAP kinases (8). The idea that MAP kinase pathways are sensitive to pressure-induced increases in wall tension is supported by the findings that p42/p44 and/or p38 MAP kinase phosphorylation increased in rat skeletal muscle arterioles exposed to pressure (Fig. 2D) (36). These results provide physiological importance to previous findings on pressure- or stretch-dependent PD-98059-sensitive increase in p42/p44 MAP kinase activity in rabbit aorta (2) and facial vein (26) and in cultured smooth muscle cells (32).

The findings that chelerythrine, PD-98059, and SB-203580 did not elicit significant decreases in smooth muscle [Ca\(^{2+}\)] (Fig. 2E), yet inhibited arteriolar myogenic tone, suggest that PKC- (1, 10, 35) and MAP kinase-dependent pathways modulate primarily the Ca\(^{2+}\) sensitivity of the myogenic mechanism (3). It is likely that Ca\(^{2+}\) sensitivity of the myogenic mechanism and development of myogenic constriction is regulated by a complex interaction of multiple signaling pathways, involving activation of RhoA/Rho kinase (3, 32, 45, 49), myosin light chain kinase, and Ca\(^{2+}\)-calmodulin-dependent protein kinase II (20).

Because the effects of PD-98059 and SB-203580 on agonist-induced and myogenic arteriolar constrictions are different, one can speculate that p42/44 and p38 MAP kinases interfere with the smooth muscle contractile apparatus and/or the myogenic mechanism at different steps of the signal transduction pathways (Fig. 2F). The importance of our findings lies in the fact that in pathophysiological conditions, alterations in PKC- and MAP kinase-dependent signal transduction may result in specific changes in arteriolar contractility (39, 43) and thus impaired regulation of peripheral resistance. Several studies have demonstrated that in hypertension an increased vascular MAP kinase activity (12, 23, 39) is associated with an upregulated arteriolar myogenic mechanism due to an increased Ca\(^{2+}\) sensitivity of the smooth muscle contractile apparatus (42) and that increases in pressure-wall tension elicit enhanced constriction of skeletal muscle arterioles (16, 42). In theory, wall tension-sensitive MAP kinase pathways are potential candidates for the upregulation of myogenic arteriolar constriction that would provide the means to normalize increased wall tension during elevations of blood pressure (22, 42). In addition, chronic increases in MAP kinase and/or PKC activity in hypertension (41) by inducing smooth muscle hypertrophy may also serve to reduce wall tension.

Collectively, the results of this study suggest that in skeletal muscle arterioles, by modulating the Ca\(^{2+}\) sensitivity of the contractile machinery, PKC is involved in both myogenic- and agonist-induced constrictions, PD-98059-sensitive p42/44 MAP kinases modulate both wall tension-dependent and ANG II-induced constrictions, whereas the SB-203580-sensitive p38 MAP kinase pathway seems to be specifically involved in the mechanotransduction of wall tension.

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REFERENCES

1. Bakker EN, Kerkhof CJ, and Sipkema P. Signal transduc-

2. Birukov KG, Lehoux S, Birukova AA, Merval R, Tkachuk VA, and Tedgui A. Increased pressure induces sustained pro-


8. Davis MJ, Wu X, Nurkiewicz TR, Kawasaki J, Davis GE, Hill MA, and Meiningier GA. Integrins and mechanotransduc-


12. Hamaguchi A, Kim S, Izumi Y, and Iwao H. Chronic activa-


