Arch Pharm Res Vol 29, No 11, 1024-1031, 2006

Archives of Pharmacal Research
http://apr.psk.or.kr

Phorbol Ester-induced Contraction Through p38 Mitogen-activated Protein Kinase is Diminished in Aortas from DOCA-Salt Hypertensive Rats

Chang-Kwon Lee, Junghwan Kim[1], Kyung-Jong Won, Hwan Myung Lee, Hyo Jin Kim, Hui Yul Roh, Hyo-Jun Park, Hwa-Sup Shin[2], Tae-Kyu Park[2], Bokyung Kim, and Sang-Mok Lee[3]

Department of Physiology, College of Medicine, Konkuk University, Chungju 380-701, Korea, [1]Department of Physical Therapy, College of Natural Science, Yongin University, Yongin 449-714, Korea, [2]Division of Life Science, College of Biomedical and Health Science, Konkuk University, Chungju 380-701, Korea, and [3]Department of Veterinary Physiology, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea

(Received August 1, 2006)

The role of mitogen-activated protein kinase (MAPK) in the decreased contractile response to phorbol ester in aortic smooth muscle strips from deoxycorticosterone acetate (DOCA)-salt hypertensive rats was examined. Norepinephrine (NE) evoked greater contractility in aortic strips from DOCA rats than in those of sham-operated rats. 12-Deoxyphorbol 13-isobutyrate (DPB) induced contraction in Ca^{2+}-free medium, which was diminished in strips from DOCA rats compared to sham-operated rats. Vasoconstrictions induced by these stimulants were inhibited by SB203580 and PD098059, inhibitors of p38 MAPK and extracellular signal-regulated kinase (ERK) 1/2, respectively, in both strips. The phosphorylation of p38 MAPK and ERK1/2 induced by NE was greater in strips from DOCA rats compared to those from sham-operated rats, and this phosphorylation was inhibited by the kinase inhibitors. DPB increased the phosphorylation of p38 MAPK and ERK1/2 in strips from both animals, and the increment of p38 MAPK phosphorylation by the stimulant was diminished in strips from DOCA rats compared to sham-operated rats. These findings suggest that the Ca^{2+}-independent contraction evoked by DPB results from the activation of MAPKs in rat aortic smooth muscle and that the attenuated contractility by DPB in DOCA rat appears to be associated with diminished p38 MAPK activity.

Key words: Phorbol ester, Mitogen-activated protein kinase, Hypertension, Vascular smooth muscle, Contraction

INTRODUCTION

Hypertension is caused by a variety of pathological changes in the neuronal, renal, and vascular control mechanisms associated with blood pressure (Cain and Khalil, 2002). Vascular smooth muscle contraction influences the control of vascular resistance and blood pressure, and its dysfunction may lead to hypertension. Vascular smooth muscle contracts in the presence of increased levels of intracellular Ca^{2+} ($[Ca^{2+}]_i$), which binds to calmodulin, a Ca^{2+}-binding protein, and results in the induced activation of myosin light chain (MLC) kinase and force (Kamm and Stull, 1985; Karaki *et al.*, 1997). It is known, however, that changes in $[Ca^{2+}]_i$ do not always parallel the level of contraction and MLC phosphorylation (Hori *et al.*, 1992). Moreover, a number of stimulants cause a further contraction under Ca^{2+}-depleted conditions in intact and membrane-permeabilized smooth muscle (Hori *et al.*, 1992; Kim *et al.*, 2003a). Several molecules, including protein kinase C (PKC) and mitogen-activated protein kinase (MAPK), have also been proposed as candidates that regulate Ca^{2+}-independent contraction (Nixon *et al.*, 1995; Lee *et al.*, 1999; Kim *et al.*, 2003b).

PKC, a family of serine/threonine kinases, consists of 12 isoforms that differ based on their cofactor dependency

Correspondence to: Bokyung Kim, Department of Physiology, College of Medicine, Konkuk University, 322 Danwol-dong, Chungju 380-701, Korea
Tae-Kyu Park, Division of Life Science, College of Biomedical and Health Science, Konkuk University, 322 Danwol-dong, Chungju 380-701, Korea
Tel: 82-43-840-3726, Fax: 82-43-840-3872
E-mail: bkkim2@kku.ac.kr

during its activation. PKC might play a role as a major mediator of smooth muscle contractions (Horowitz *et al.*, 1996; Kim *et al.*, 2003b). PKC is known to activate several cellular signaling pathways including ion channels and MLC (Li *et al.*, 1998; Kamp and Hell, 2000). The activation of PKC induces and elevates vascular smooth muscle contraction, even in the absence of extracellular Ca^{2+} (Walsh *et al.*, 1996). It has been reported that PKC inhibits myosin phosphatase, which could be the mechanism underlying the PKC-mediated increase in Ca^{2+}-sensitivity (Kitazawa *et al.*, 2000; Sakamoto *et al.*, 2003).

The MAPKs are a family of serine/threonine-specific protein kinases that includes extracellular signal-regulated kinase (ERK) 1/2, p38 MAPK, and stress-activated protein kinase/c-*Jun* NH_2-terminal kinase (SAPK/JNK) (Miyamoto *et al.*, 1999; Widmann *et al.*, 1999). In vascular smooth muscle, MAPKs are known to be activated by receptor agonists (Dessy *et al.*, 1998; Touyz *et al.*, 1999). A number of studies have reported that the MAPK pathway is closely associated with the increase in smooth muscle contraction observed under Ca^{2+}-dependent and -independent conditions (Dessy *et al.*,1998; Kwon *et al.*, 2003). The inhibition of p38 MAPK attenuates contractility in smooth muscle cells, suggesting that p38 MAPK contributes to the elevation of contraction.

In deoxycorticosterone acetate (DOCA)-salt hypertensive rats, the altered reactivity of blood vessels is often associated with an elevation of systolic blood pressure (Watts, 1998). The contraction of vascular smooth muscle by vasoconstrictors, including 5-hydroxytryptamine and norepinephrine (NE), is significantly increased in hypertensive animals (Watts, 1998; Touyz *et al.*, 1999; Tosters *et al.*, 2000). We recently reported that the activation of MAPK is essential for the agonist-mediated contraction in vascular smooth muscle in DOCA-salt hypertensive rats; this contraction is not inhibited by the chelation of extracellular Ca^{2+}, implying that MAPK is involved in vasoconstriction through a Ca^{2+}-independent pathway (Kim *et al.*, 2004a; Kim *et al.*, 2005). Although it is known that contractile responsiveness to receptor agonists is mediated by p38 MAPK, the mechanism is not clear in the case of DOCA-salt hypertension. Moreover, it has been reported that MAPK activity is regulated by PKC in vascular smooth muscle (Khalil *et al.*, 1992; Khalil and Morgan, 1993; Liao *et al.*, 1997). However, the mechanism relating MAPK and PKC, especially in the regulation of vascular contraction, remains unclear. In this study, therefore, we determined the relationship between PKC and MAPK in contraction and examined the roles of MAPK, especially p38 MAPK, in the contractile response to phorbol ester between sham-operated and DOCA-salt hypertensive rats.

MATERIALS AND METHODS

Animal models for hypertension

Animal care and all experiments were conducted in accordance with the institutional guidelines of Konkuk University, South Korea. We used male Sprague-Dawley rats (6 weeks old, 180-190 g) purchased from Daehan-biolink (Chungju, South Korea). Both DOCA-salt hypertensive and sham-operated rats (control) were prepared as previously reported (Kim *et al.*, 2004a). Briefly, animals were uninephrectomized, and, after 1 week, received a subcutaneous silicon rubber implant impregnated with DOCA (200 mg/kg) under intramuscular anesthesia (35 mg/kg ketamine plus 5 mg/kg xyalzine). DOCA rats received a 0.9% NaCl plus 0.2% KCl drinking solution. Controls were also operated on without receiving an implant and thereafter received normal tap water. All animals were fed standard laboratory rat chow with *ad libitum* access to both food and water. Systolic blood pressure was directly measured with a pressure transducer (Statham P23XL Viggo Spectramed, Oxnard, CA, USA) at the common carotid artery under anesthesia (as above).

Tissue preparation

The animals were sacrificed using CO_2 gas and bled rapidly by cutting the carotid arteries. The thoracic aorta was rapidly and carefully removed and placed in physiological salts solution (PSS), which contained (in mM): NaCl 136.9; KCl 5.4; $CaCl_2$ 1.5; $MgCl_2$ 1.0; $NaHCO_3$ 23.8; EDTA 0.01. The aorta was dissected free of fat and connective tissue, and cut into strips (2-3 mm wide and 5-6 mm in length). The endothelium was removed by gently rubbing the inner surface of the vessel with cotton balls soaked in PSS.

Measurement of isometric contraction

The prepared strips were vertically suspended in 5 mL organ baths. One end of each strip was attached to a stainless steel rod, and the other to a force transducer (FT03; Grass-Telefactor Instruments, West Warwick, RI, U.S.A.). Changes in muscle force were isometrically recorded on Grass 79E polygraphs. After the strips were suspended under resting tension of 10 mN, they were equilibrated for 20 min in an organ bath filled with PSS and sequentially exposed to 70 mM KCl and PSS three times. The high KCl solution was prepared by replacing NaCl with an equimolar amount KCl. All bath solutions were saturated with a mixture of 95% O_2 and 5% CO_2 at pH 7.4 and maintained at 37°C.

Measurement of protein phosphorylation

Aortic strips were isolated and snap-frozen in liquid N_2

after treatment with various stimulants for different times. The samples were then homogenized in sample buffer containing 50 mM Tris-HCl (pH 7.4), 5 mM EGTA, 5 mM dithiothreitol, 300 μM phenylmethyl sulfonyl fluoride, 20 mM β-glycerophosphate, 1 mM NaF, 2 mM Na_3VO_4, 5 μg/mL aprotinin, 5 μM leupeptin, 1% Triton X-100, 10% glycerol, and 150 mM NaCl. The homogenate was centrifuged at 14,000 × *g* for 10 min at 4°C, and the supernatant was collected (Kim *et al.*, 2003b). Protein concentrations were determined using Bio-Rad DC protein assay reagents (Bio-Rad, Hercules, CA, U.S.A.), a colorimetric assay for protein based on the Lowry assay. The protein homogenates were diluted 1:1 (v/v) with sodium dodecyl sulfate (SDS) sample buffer containing 40 mM Tris-HCl (pH 6.8), 8 mM EGTA, 4% 2-mercaptoethanol, 40% glycerol, 0.01% bromophenol blue, and 4% SDS, and then boiled for 5 min.

Equivalent amounts (30-50 μg/lane) of aortic protein were separated on 10% SDS-PAGE gels. After transferring the separated proteins to a polyvinylidene fluoride membrane (Millipore, Bedford, MA, U.S.A.), they were blocked for 60 min with phosphate-buffered saline (PBS) containing 5% non-fat dried milk to prevent non-specific binding. Thereafter, the membranes were incubated with PBS and 0.05% Tween-20 and with individual antibodies diluted 1:1000 to 1:5000 overnight at 4°C. Following incubation with horseradish peroxidase-conjugated anti-rabbit IgG (1:1000) for 60 min, the blots were developed using an enhanced chemiluminescence detection system (Amersham-Pharmacia, Piscataway, NJ, U.S.A.). Antibody-specific bands were quantified using an image analyzer (Bio-Rad, Hercules, CA, U.S.A.).

Materials

12-Deoxyphorbol 13-isobutyrate (DPB) was purchased from Funakoshi (Japan).

DOCA and NE were purchased from Sigma (St Louis, MO, U.S.A.). SB203580 and PD098059 were purchased from Tocris (Ellisville, MO, U.S.A.). Polyclonal anti-phosphorylated and -nonphosphorylated ERK1/2 antibodies, Triton-X 100, and dithiothreitol were purchased from Promega (Madison, WI, U.S.A.). Polyclonal anti-phosphorylated and -nonphosphorylated p38 MAPK antibodies were purchased from Cell Signaling (Beverly, MA, U.S.A.). Glycine and Tween 20 were purchased from Bio-Rad. Bromophenol blue and the low molecular weight electrophoresis calibration kit were purchased from Amersham-Pharmacia (U.S.A.). Ketamine and xylazine were purchased from Yuhan (South Korea) and Bayer (South Korea), respectively.

Data analysis

Data are presented as the means ± standard errors of the mean (S.E.M). Unpaired Student's *t*-tests were used to compare the data, and a *P* value of < 0.05 was considered significantly different.

RESULTS

The changes in body and organ weights and blood pressure in DOCA rats

The relative weights of heart and kidney were significantly elevated in the hypertensive rats compared to sham-operated normotensive controls (Fig. 1A and 1B). Total body weight was decreased in DOCA-salt hypertensive rats compared to sham-operated rats (Fig. 1C). Blood pressure, which was higher in DOCA-salt hypertensive rats than in sham-operated rats, showed a weakly dependent increase up to 4 weeks and thereafter decreased slightly (Fig. 1D).

Agonist-mediated vasoconstrictions

NE (0.1 nM-100 μM) induced contraction in a dose-dependent manner in aortic strips isolated from both sham-operated controls and DOCA-salt hypertensive rats. The contraction of the strip induced by NE was significantly greater in the hypertensive rats compared to the sham-operated controls (Fig. 2A). DPB (1 nM-10 μM) in Ca^{2+}-free PSS containing 1 mM EGTA also induced con-

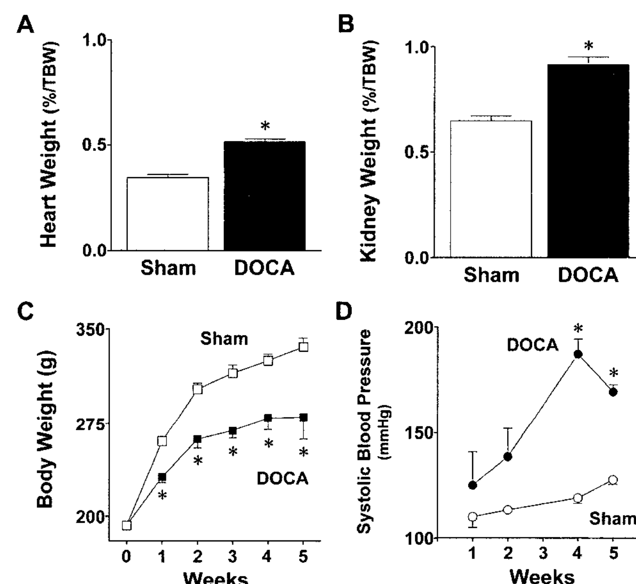

Fig. 1. Comparison of organ weight, body weight, and systolic blood pressure in sham-operated and DOCA-salt hypertensive rat. Weights of heart (A) and kidney (B), total body weight (C), and systolic blood pressure (D) were determined in sham-operated and DOCA-salt hypertensive rats. The gain in organ weight was measured at 4 weeks after the silicon rubber implantation, and is expressed as the % of total body weight (%/ TBW). Each data-point represents the mean ± S.E.M. (n= 15 to 28). *Significantly different from sham-operated rats with *P* < 0.05.

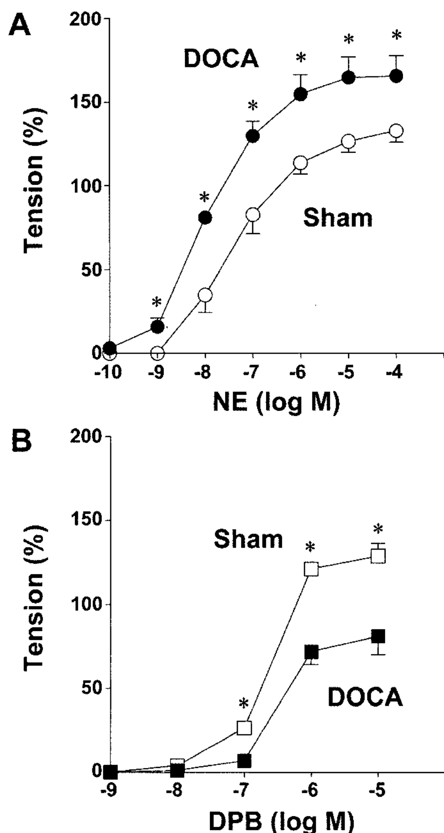

Fig. 2. Concentration-response curve for contractions induced by NE (A) and DPB (B) in aortic smooth muscle strips from sham-operated and DOCA-salt hypertensive rat. Strips were treated cumulatively with NE (0.1 nM-100 μM) in normal PSS or DPB (1 nM-10 μM) in Ca^{2+}-free PSS containing 1 mM EGTA. The muscle contraction induced by 70 mM high K^+ obtained just before starting the experiments was taken as 100%. Each data point represents the mean ± S.E.M ($n = 6$ to 11). *Significantly different from sham-operated rats with $P < 0.05$.

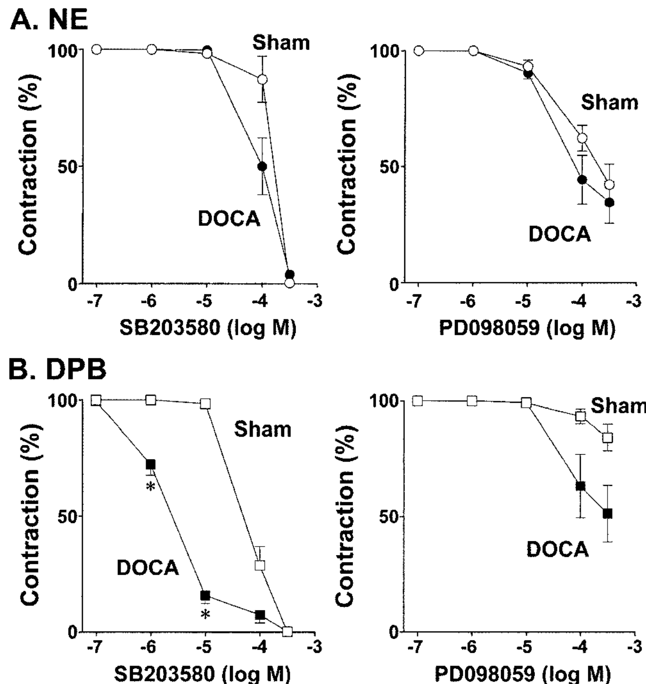

Fig. 3. Effect of MAPK inhibitors on contraction evoked by agonists in aortic smooth muscle strips from sham-operated and DOCA-salt hypertensive rats. Aortic strips were precontracted with 10 μM NE (A) in normal PSS or 1 μM DPB (B) in Ca^{2+}-free PSS, and then treated with SB203580 or PD098059 cumulatively. The contractions induced by NE and DPB before treatment with the inhibitors were taken as 100%. Results were expressed as mean ± S.E.M (n= 4 to 7). *Significantly different from sham-operated rats with $P < 0.05$.

traction in aortic strips from both sham-operated controls and from DOCA rats. However, the contraction of aortic strips generated by DPB was significantly smaller in the hypertensive rats than in sham-operated controls (Fig. 2B).

Effect of kinase inhibitors on agonist-mediated vasoconstriction

To determine the role of p38 MAPK in phorbol ester-induced contraction, SB203580, an inhibitor of p38 MAPK, was tested in DPB-induced aortic strip contraction. SB203580 (0.1-300 μM) inhibited contraction induced by NE (10 μM) in a dose-dependent manner (Fig. 3A). SB203580 (0.1-300 μM) also inhibited DPB (1 mM)-mediated contraction in a dose-dependent manner; DPB-mediated contraction was significantly greater in DOCA-salt hypertensive rats compared to sham-operated rats (Fig. 3B).

Furthermore, we also examined the effects of PD098059, an inhibitor of ERK1/2, on DPB-induced aortic strip contraction in order to evaluate the association between ERK1/2 kinase and phorbol ester-induced contraction. As shown in Fig. 3, PD098059 (0.1-300 μM) generated a dose-dependent decrease in contraction induced by 10 mM NE (A) and 1 mM DPB (B); this contraction by the maximal concentration of both sets of agonists in both types of animal was not completely abolished by PD098059. On the other hand, the inhibition of the induced contraction by PD098059 did not differ between DOCA-salt hypertensive and sham-operated rats (Fig. 3A and B)

Activity of MAPK induced by agonists

We next measured the reactivity of MAPK with phosphorylated MAPK antibodies, to determine the role of MAPK in receptor agonist-induced responses in aortic strips from sham-operated and DOCA-hypertensive rats. In the quiescent state without any stimulant, the phosphorylation of p38 MAPK was significantly increased in DOCA-salt hypertensive rats compared to sham-operated rats. NE (10 μM) caused an increase in the level of phosphorylation of p38 MAPK in both strips (Fig. 4A). The magnitude of phosphorylation of p38 MAPK induced by

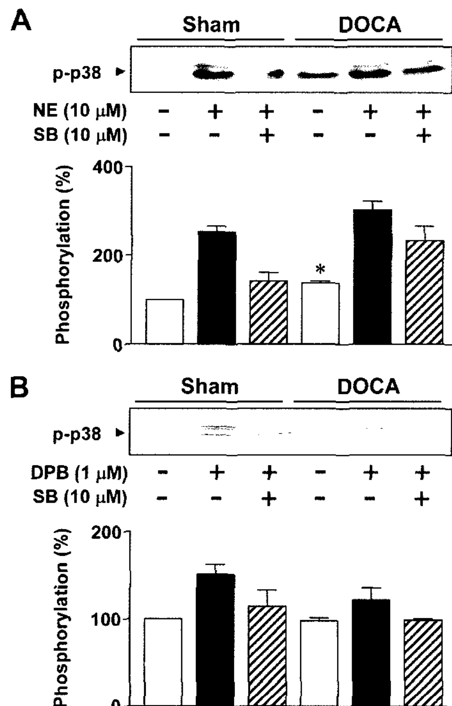

Fig. 4. Effect of SB203580 on p38 MAPK phosphorylation stimulated by agonists in aortic smooth muscle strips from sham-operated and DOCA-salt hypertensive rats. Aortic strips were pretreated with SB203580 (10 μM for 15 min) and then stimulated with 10 μM NE (A) for 15 min or 1 μM DPB (B) in Ca^{2+}-free PSS for 20 min. The p38 MAPK phosphorylation levels were determined by immunoblotting using an anti-phosphorylated p38 MAPK antibody. In A and B, the upper panel displays representative blots of phosphorylated p38 MAPK and the lower panel shows a summary of the densitometric results. Basal level of phosphorylated p38 MAPK in sham-operated rats is expressed as 100%. Each result represents the mean ± S.E.M (n=4). *Significantly different from sham-operated rats with $P < 0.05$. SB, SB203580. p-p38, phosphorylated p38 MAPK.

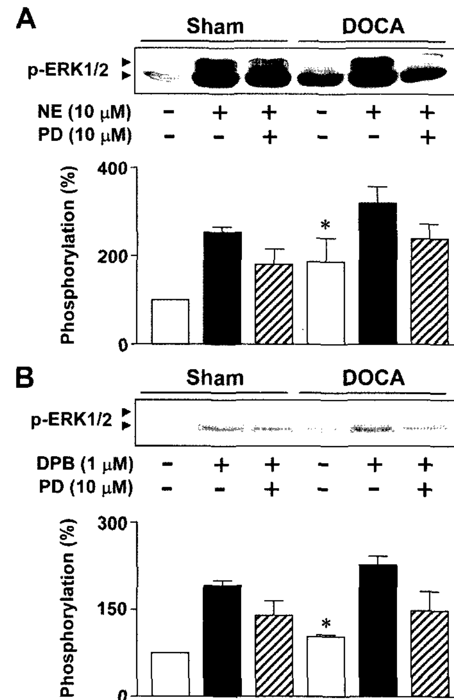

Fig. 5. Effect of PD098059 on ERK1/2 phosphorylation generated by agonists in aortic smooth muscle strips from sham-operated and DOCA-salt hypertensive rats. The aortic strips were preincubated with PD098059 (10 μM for 15 min) and then stimulated with 10 μM NE (A) for 15 min or 1 μM DPB (B) in Ca^{2+}-free PSS for 20 min. Levels of ERK1/2 phosphorylation were examined by immunoblotting using an anti-phosphorylated ERK1/2 antibody. In A and B, representative blots of phosphorylated ERK1/2 are displayed in the upper panel and densitometric results are summarized in the lower panel. Basal level of phosphorylated ERK1/2 in sham-operated rats is expressed as 100%. Each result represents the mean ± S.E.M (n= 4 in each experiment). *Significantly different from sham-operated rats with $P < 0.05$. PD, PD098059. p-ERK1/2, phosphorylated ERK1/2.

NE was greater in DOCA-salt hypertensive rats compared to sham-operated rats and was inhibited by the addition of SB203580 (10 μM) (Fig. 4A). DPB (1 μM) in Ca^{2+}-free medium containing 1 mM EGTA increased the phosphorylation of p38 MAPK in strips from sham-operated and DOCA-salt hypertensive rats; this phosphorylation was also inhibited by SB203580 (10 μM). However, the level of phosphorylated p38 MAPK was greater in strips from sham-operated rats than in those from DOCA-salt hypertensive rats (Fig. 4B). The total expression of p38 MAPK using nonphosphorylated p38 MAPK antibody was not changed (data not shown).

We also tested the activity of ERK1/2 for receptor agonist-induced responses in aortic strips from sham-operated and DOCA-salt hypertensive rats. As shown in Fig. 5, in the quiescent state, the phosphorylation of ERK1/2 increased significantly in DOCA-salt hypertensive rats compared to sham-operated rats. The NE (10 μM)-induced phosphorylation of ERK1/2 increased in both animals, and this responsiveness was greater in DOCA-salt hypertensive rat compared to sham-operated controls. PD098059 (10 μM) decreased NE (10 μM)-induced phosphorylation of ERK1/2 in both animals (Fig. 5A). Similarly, DPB (1 μM) also induced increased phosphorylation of ERK1/2 in Ca^{2+}-free medium containing 1 mM EGTA in both DOCA-salt hypertensive and sham-operated rats that was attenuated by 10 μM PD098059 (Fig. 5B). No difference in the magnitude of ERK1/2 phosphorylation induced by DPB was observed between DOCA-salt hypertensive and sham-operated rats (Fig. 5B). The total expression of ERK1/2 using nonphosphorylated ERK1/2 antibody was not changed (data not shown).

DISCUSSION

In the present study, we showed that phorbol ester, a

PKC activator, evoked a dose-dependent contraction in aortic strips in Ca^{2+}-free medium containing EGTA from both DOCA-salt hypertensive and sham-operated rats. Moreover, this Ca^{2+}-independent vascular contraction was strongly inhibited by a p38 MAPK inhibitor. These results imply that the MAPK pathway, especially p38 MAPK, is activated by the PKC-mediated pathway and plays a significant role in the regulation of aortic smooth muscle contraction. It is known that MAPK can be activated by Ca^{2+}-dependent and -independent isoforms of PKC (Liao *et al.*, 1997) and that PKC can be upstream in the MAPK pathway, which is associated with the increment in basal tone in hypertensive rats (Kim *et al.*, 2005). Moreover, Ca^{2+}-independent contraction is dependent on PKC activity (Khalil *et al.*, 1992) and is also associated with MAPK stimulation (Khalil and Morgan, 1993) in vascular smooth muscle. We previously reported that MAPK contributes to vascular contractions that are not regulated by extracellular Ca^{2+} (Park *et al.*, 2003). The current results, therefore, suggest that phorbol ester-induced Ca^{2+}-independent contractions may well be mediated by the p38 MAPK pathway.

The contraction and phosphorylation of MAPK was elevated by NE and occurred in both DOCA-salt hypertensive and sham-operated rats. Furthermore, the p38 MAPK inhibitor decreased the contraction and activity of MAPK elevated by NE in both sham-operated and DOCA-salt hypertensive rats. In the present study, the level of MAPK phosphorylation in the quiescent state was significantly increased in muscle strips from DOCA-salt hypertensive rats compared to those from sham-operated rats. A similar result was obtained in the measurement of MAPK activity. These results are consistent with previous reports on aortic smooth muscle of hypertensive rats (Kim *et al.*, 2004b; Kwon *et al.*, 2004; Kim *et al.*, 2005). In contrast to the results for NE, the contractile response to phorbol ester was significantly attenuated in DOCA-salt hypertensive rats compared to sham-operated rats. Similar to the results of the mechanical study, the phosphorylation of p38 MAPK, but not ERK1/2, by phorbol ester was diminished in hypertensive rats. The findings herein show that the contractile response and p38 MAPK phosphorylation by ET-1 was significantly attenuated in strips from DOCA-salt hypertensive rats compared to sham-operated controls and that this attenuated response to the agonist may result from reduced p38 MAPK activity in DOCA-salt hypertensive rats (Kim *et al.*, 2005). It has been reported that ET-1 induces the contraction and activation of PKC in vascular smooth muscle (McNair *et al.*, 2004; Sirous *et al.*, 2004). Therefore, it can be speculated that the diminished response to ET-1 may be mediated by PKC, which stimulates the p38 MAPK pathway.

Elevated blood pressure leads to morphological and functional changes in blood vessels (Kobayashi *et al.*, 2003). The increment of vascular responsiveness to vasoconstrictors is often related to an elevation in systolic blood pressure. The vascular contractility by receptor agonists is significantly increased in hypertensive rats (Dessy *et al.*, 1998; Touyz *et al.*, 1999; Kim *et al.*, 2005). In our previous study, we reported that the activity of MAPK and *h*-caldesmon phosphorylation by agonists is increased in aortic strips from sham-operated and DOCA-salt hypertensive rats, in which the responsiveness was inhibited by an inhibitor of MAPK (Kim *et al.*, 2005; Lee *et al.*, 2006). Those studies suggest that the MAPK pathway may induce the phosphorylation of caldesmon, thus contracting aortic smooth muscle in DOCA-salt hypertensive rats. Moreover, Multhalif and coworkers (2000) reported that the systolic blood pressure in hypertensive rats is decreased by a MAPK inhibitor. This, therefore, suggests that the increased MAPK activity may be a cause of the increased blood pressure in DOCA-salt hypertensive rats. In the present study, on the other hand, we found that DOCA-salt hypertensive rat showed a decrease in MAPK phosphorylation and contractility of aortic strips induced by DPB compared to sham-operated controls. These results, therefore, suggest that DOCA-salt hypertension may lead to attenuated MAPK activity in aortic smooth muscle, shown in the diminished responsiveness to a phorbol ester, in order to compensate for the increase in vascular resistance and blood pressure that occurs during DOCA-salt hypertension.

In conclusion, the findings reported herein confirm that the MAPK pathway could play a key role in the contractile process induced by phorbol ester and NE in vascular smooth muscle. Moreover, the attenuated contractility evoked by phorbol ester in DOCA-salt hypertensive rats may originate from a diminution in p38 MAPK activity.

ACKNOWLEDGEMENTS

This work was supported by grant from the Bio-Food & Drug Research Center at Konkuk University and the Regional Research Centers Program of the Ministry of Education & Human Resources Development in Korea.

REFERENCES

Cain, A.E. and Khalil, R.A., Pathophysiology of essential hypertension: role of the pump, the vessel, and the kidney. *Semin. Nephrol.*, 22, 3-16 (2002).

Dessy, C., Kim, I., Sougnez, C.L., Laporte, R., and Morgan, K.G., A role for MAP kinase in differentiated smooth muscle contraction evoked by adrenoceptor stimulation. *Am. J. Physiol.*, 275, C1081-C1086 (1998).

Fu, X., Gong, M.C., Jia, T., Somlyo, A.V., and Somlyo, A.P., The effects of the Rho-kinase inhibitor Y-27632 on arachidonic

acid-, GTPãS-, and phorbol ester-induced Ca^{2+}-sensitization of smooth muscle. *FEBS Lett.*, 440, 183-187 (1998).

Hori, M., Sato, K., Sakata, K., Ozaki, H., Takano-Ohmuro, H., Tsuchiya, T., Sugi, H., Kato, I., and Karaki, H., Receptor agonists induce myosin phosphorylation-dependent and phosphorylation-independent contractions in vascular smooth muscle. *J. Pharmacol. Exp. Ther.*, 261, 506-512 (1992).

Horowitz, A., Clement-Chomienne, O., Walsh, M.P., and Morgan, K.G., Epsilon-isoenzyme of protein kinase C induces a Ca^{2+}-independent contraction in vascular smooth muscle. *Am. J. Physiol.*, 271, C589-C594 (1996).

Kamm, K.E. and Stull, J.T., The function of myosin and myosin light chain kinase phosphorylation in smooth muscle. *Annu. Rev. Pharmacol. Toxicol.*, 25, 593-620 (1985).

Kamp, T.J. and Hell, J.W., Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. *Circ. Res.*, 87, 1095-102 (2000).

Karaki, H., Ozaki, H., Hori, M., Mitsui-Saito, M., Amano, K., Harada, K., Miyamoto, S., Nakazawa, H., Won, K.J., and Sato, K., Calcium movements, distribution, and functions in smooth muscle. *Pharmacol. Rev.*, 49, 157-230 (1997).

Khalil, R.A., Lajoie, C., Resnick, M.S., and Morgan, K.G., Ca^{2+}-independent isoforms of protein kinase C differentially translocate in smooth muscle. *Am. J. Physiol.*, 263, C714-C719 (1992).

Khalil, R.A. and Morgan, K.G., PKC-mediated redistribution of mitogen-activated protein kinase during smooth muscle cell activation. *Am. J. Physiol.*, 265, C406-C411 (1993).

Kim, B., Kim, Y.S., Ahn, J., Kim, J., Cho, S.I., Won, K.J., Ozaki, H., Karaki, H., and Lee, S., Conventional-type protein kinase C contributes to phorbol ester-induced inhibition of rat myometrial tension. *Br. J. Pharmacol.*, 139, 408-414 (2003b).

Kim, B., Kim, J., Bae, Y.M., Cho, S.I., Kwon, S.C., Jung, J.Y., Park, J.C., and Ahn, H.Y., p38 Mitogen-activated protein kinase contributes to the diminished aortic contraction by endothelin-1 in DOCA-salt hypertensive rats. *Hypertension,* 43, 1086-1091 (2004a).

Kim, Y.S., Kim, B., Karaki, H., Hori, M., and Ozaki, H., Up-regulation of Rnd1 during pregnancy serves as a negative-feedback control for Ca^{2+} sensitivity of contractile elements in rat myometrium. *Biochem. Biophys. Res. Commun.*, 311, 972-978 (2003a).

Kim, B., Kim, J., Kim, A., Kim, Y.S., Lee, Y.R., Bae, Y.M., Cho, S., and Ryu, M.R., Ligusticum wallichi-induced vasorelaxation mediated by mitogen-activated protein kinase in rat aortic smooth muscle. *J. Ethnopharmacol.*, 90, 397-401 (2004b).

Kim, J., Lee, Y.R., Lee, C.H., Choi, W.H., Lee, C.K., Kim, J., Bae, Y.M., Cho, S., and Kim, B., Mitogen-activated protein kinase contributes to elevated basal tone in aortic smooth muscle from hypertensive rats. *Eur. J. Pharmacol.*, 514, 209-215 (2005).

Kitazawa, T., Eto, M., Woodsome, T.P., and Brautigan, D.L., Agonists trigger G protein-mediated activation of the CPI-17 inhibitor phosphoprotein of myosin light chain phosphatase to enhance vascular smooth muscle contractility. *J. Biol. Chem.*, 275, 9897-9900 (2000).

Kobayashi, N., Mita, S., Yoshida, K., Honda, T., Kobayashi, T., Hara, K., Nakanou, S., Tsubokou, Y., and Matsuoka, H., Celiprorol activates eNOS through the PI3K-Act pathway and inhibits VCAM-1 via NF-êB induced by oxidative stress. *Hypertension*, 42: 1004-1013 (2003).

Kwon, S., Lee, W.J., Fang, L.H., Kim, B., and Ahn, H.Y., Mitogen-activated protein kinases partially regulate endothelin-1-induced contractions through a myosin light chain phosphorylation-independent pathway. *J. Vet. Med. Sci.*, 65, 225-230 (2003).

Kwon, S., Fang, L.H., Kim, B., Ha, T.S., Lee, S.J., and Ahn, H.Y., p38 Mitogen-activated protein kinase regulates vasoconstriction in spontaneously hypertensive rats. *J. Pharmacol. Sci.*, 95, 267-272 (2004).

Lee, Y.H., Kim, I., Laporte, R., Walsh, M.P., and Morgan, K.G., Isozyme-specific inhibitors of protein kinase C translocation: effects on contractility single permeabilized vascular muscle cells of the ferret. *J. Physiol.*, 517, 709-720 (1999).

Lee, Y.R., Lee, C.K., Park, H.J., Kim, H., Kim, J., Kim, J., Lee, K.S., Lee, Y.L., Min, K.O., and Kim, B., c-Jun N-terminal kinase contributes to norepinephrine-induced contraction through phosphorylation of caldesmon in rat aortic smooth muscle. *J. Pharmacol. Sci.*, 100, 119-125 (2006).

Li, L., Eto, M., Lee, M.R., Morita, F., Yazawa, M., and Kitazawa, T., Possible involvement of the novel CPI-17 protein in protein kinase C signal transduction of rabbit arterial smooth muscle. *J. Physiol.*, 508, 871-881 (1998).

Liao, D.F., Monia, B., Dean, N., and Berk, B.C., Protein kinase C- mediates angiotensin II activation of ERK1/2 in vascular smooth muscle cells. *J. Biol. Chem.*, 272, 6146-6150 (1997).

McNair, L.L., Salamanca, D.A., and Khalil, R.A., Endothelin-1 promotes Ca^{2+} antagonist-insensitive coronary smooth muscle contraction via activation of å-protein kinase C. *Hypertension*, 43, 897-904 (2004).

Miyata, Y. and Nishida, E., Distantly related cousins of MAP kinase: biochemical properties and possible physiological functions. *Biochem. Biophys. Res. Commun.*, 266, 291-295 (1999).

Multhalif, M.M., Benter, I.F., Khandekar, Z., Gaber, L., Estes, A., Malik, S., Parmentier, J.H., Manne, V., and Malik, K.U., Contribution of Ras GTPase/MAP kinase and cytochrome P450 metabolites to deoxycorticosterone-salt induced hypertension. *Hypertension*, 35, 457-463 (2000).

Nixon, G.F., Iizuka, K., Haystead, C.M., Haystead, T.A., Somlyo, A.P., and Somlyo, A.V., Phosphorylation of caldesmon by mitogen-activated protein kinase with no effect on Ca^{2+} sensitivity I rabbit smooth muscle. *J. Physiol.*, 487, 283-289 (1995).

Park, S., Kim, B., Kim, J., Won, K.J., Lee, S., Kwon, S., and Cho, S., Tamoxifen induces vasorelaxation via inhibition of mitogen-activated protein kinase in rat aortic smooth muscle. *J. Vet. Med. Sci.*, 65, 1155-1160 (2003).

Sakamoto, K., Hori, M., Izumi, M., Oka, T., Kohama, K., Ozaki, H., and Karaki, H., Inhibition of high K^+-induced contraction by the ROCKs inhibitor Y-27632 in vascular smooth muscle: possible involvement of ROCKs in a signal transduction pathway. *J. Pharmacol. Sci.*, 92, 56-69 (2003).

Sirous, Z.N., Fleming, J.B., and Khalil, R.A., Endothelin-1 enhances eicosanoids-induced coronary smooth muscle contraction by activating specific protein kinase C isoforms. *Hypertension*, 37, 497-504 (2001).

Taggart, M.J., Lee, Y.H., and Morgan, K.G. Cellular redistribution of PKC, rhoA, and ROK following smooth muscle agonist stimulation. *Exp. Cell. Res.*, 25, 92-101 (1999).

Tostes, R.C., David, F.L., Carvalho, M.H., Nigro, D., Scivoletto, R., and Fortes, Z.B., Gender differences in vascular reactivity to endothelin-1 in deoxycorticosterone-salt hypertensive rats. *J. Cardiovasc. Pharmacol.*, 36, S99-S101 (2000).

Touyz, R.M., El Mabrouk, M., He, G., Wu, X.H., and Schiffrin, E.L., Mitogen-activated protein/extracellular signal-regulated kinase inhibition attenuates angiotensin II-mediated signaling and contraction in spontaneously hypertensive rat vascular smooth muscle cells. *Circ. Res.*, 84, 505-515 (1999).

Walsh, M.P., Horowitz, A., Clement-Chomienne, O., Andrea, J.E., Allen, B.G., and Morgan KG., Protein kinase C mediation of Ca^{2+}-independent contractions of vascular smooth muscle. *Biochem. Cell Biol.*, 74, 485-502 (1996).

Watts, S.W., The development of enhanced arterial serotonergic hyper-responsiveness in mineralocorticoid hypertension. *J. Hypertens.*, 16, 811-822 (1998).

Widmann, C., Gibson, S., Jarpe, M.B., and Johnson, G.L., Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol. Rev.*, 79, 143-180 (1999).