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Mitogen-activated protein kinase contributes to elevated basal tone in aortic smooth muscle from hypertensive rats

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Abstract

The role of mitogen-activated protein kinase (MAPK) in increased basal tone –spontaneous resistance in vascular muscle strips– was clarified in aortic smooth muscle from deoxycorticosterone acetate (DOCA)-salt hypertensive rats. The MAPK/extracellular signal-regulated protein kinase (ERK) kinase inhibitor, PD098059 (2′-amino-3′-methoxyflavone), significantly inhibited basal tone in a dose-dependent manner. The basal level of ERK1/2 activation was inhibited by PD098059 and was significantly greater in hypertensive rats than in sham-operated rats. In contrast, inhibition with PD098059 was not observed in sham-operated rats. GF109203X (2-[1-(3-dimethylaminopropyl)-1*H*-indol-3-yl]-3-(1*H*-indol-3-yl)maleimide), an inhibitor of protein kinase C (PKC), decreased both basal tone and ERK1/2 activity in the hypertensive rats. In contrast, Y27632 ((*R*)-(+)-*trans*-*N*-(4-Pyridyl)-4-(1-aminoethyl)cyclohexanecarboxamide) and verapamil, inhibitors of Rho kinase and voltage-dependent Ca^{2+} channels, respectively, significantly inhibited basal tone but not ERK1/2 activity. Thus, basal vascular tone is elevated by the altered activation of MAPK in DOCA-salt hypertensive rats, and this is regulated by PKC, but not by Rho or intracellular Ca^{2+}.

Keywords: Hypertension; Basal vascular tone; Mitogen-activated protein kinase; Vascular smooth muscle; Protein kinase C

1. Introduction

Increased blood pressure during hypertension is associated with several changes in the physiological characters of the vessels. These changes often occur in medial smooth muscle, which is an important effector in the regulation of vasoconstriction (Epstein et al., 1997). Total peripheral resistance comprises a summation of vascular reactivity to contractile agonists, and basal tone –spontaneous resistance in vascular muscle strips– is increased in patients and experimental animal models with essential and secondary hypertension (Ghosh et al., 2004; Krum et al., 1998). It is well established that vascular smooth muscle contraction is regulated by intracellular Ca^{2+} ($[Ca^{2+}]_i$) and by the phosphorylation of myosin light chain (Somlyo and Himpens, 1989). The increased $[Ca^{2+}]_i$ can phosphorylate the 20 kDa myosin light chain by the activation of myosin light chain kinase, which initiates vasoconstriction. In addition to the $[Ca^{2+}]_i$-myosin phosphorylation pathway, a number of intracellular signal molecules, including mitogen-activated protein kinase (MAPK), protein kinase C (PKC), phosphatidylinositol 3 kinase (PI3K), and low molecular GTP-binding Rho protein, are believed to play important roles in the regulation of vascular smooth muscle contraction (Kim et al., 2003).

MAPK is a widespread intracellular protein kinase that acts in the regulation of cell function (Stoclet et al., 2004). Its family of enzymes includes extracellular signal-regulated protein kinase (ERK) 1/2, p38 MAPK, and stress-activated protein kinase/c-Jun NH_2-terminal kinase (SAPK/JNK).

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MAPK can be activated by a variety of stimuli, including mitogens, growth factors, hormones, oxidants and environmental stress factors. Although MAPK activation is generally involved in cell growth and proliferation, this pathway may also play an important role in vascular smooth muscle contraction (Dessy et al., 1998; Touyz et al., 1999). We have reported that activation of MAPK is essential for the agonist-mediated contraction in vascular smooth muscle from deoxycorticosterone acetate (DOCA)-salt hypertensive rats (Kim et al., 2004).

Myogenic basal tone depends on $[Ca^{2+}]_i$ in smooth muscle cells, which is increased in hypertensive vessels (Zou et al., 1995). PKC also modulates basal vascular tone (Osol et al., 1991). In addition, the increased basal tone in hypertensive rats is also regulated by PI3K- and Rho/Rho kinase-mediated pathways (Northcott et al., 2004; Wehrwein et al., 2004). Both PKC and PI3K can phosphorylate, and subsequently activate MAPK in muscle cells (Adam et al., 1995). Although these results suggest that MAPK may be involved in the elevation of basal tone, to our knowledge there is no study showing direct evidence of the involvement of MAPK in elevated basal vascular tone in hypertensive rats. We therefore studied this question in DOCA-salt hypertensive rats.

2. Materials and methods

2.1. Animal models of hypertension

Male Sprague–Dawley rats (6 weeks old, 180–190 g) were obtained from Daehanbiolink Co. Ltd. (Choongju, Korea). Animals were maintained at a constant temperature of 21±2 °C and 55±8% relative humidity on a normal 12–12 h light–dark cycle. All experiments were performed in accordance with the institutional guidelines of Konkuk University, Korea. Animals were separated into groups of sham-operated and DOCA-salt hypertensive rats. Animals underwent uninephrectomy under intramuscular anaesthesia (35 mg/kg ketamine plus 5 mg/kg xylazine). The adrenal glands in both groups were left intact, because adrenalectomy prevents the development of hypertension. After 1 week, a silastic rubber impregnated with DOCA (200 mg/kg) was implanted subcutaneously in the subscapular region of the rats under anaesthesia. Controls (sham-operated rats) were also operated on without receiving an implant. After surgery, the DOCA-salt hypertensive rat received 0.9% NaCl plus 0.2% KCl drinking solution. Sham-operated rats received normal tap water. All animals were fed standard laboratory rat chow and had ad libitum access to both food and water. Systolic blood pressure was measured by a direct method under anaesthesia (as above). The left common carotid artery was cannulated and connected to a physiological pressure transducer (Statham P23XL Viggo Spectramed, Oxnard, CA, USA). After a 15-min equilibration period, systolic blood pressure was measured using Grass 79E polygraphs (Grass-Telefactor, West Warwick, RI, USA).

2.2. Tissue preparation

The animals were stunned and bleed, and rapidly exsanguinated by cutting the carotid arteries. The thoracic aorta was carefully removed and placed in physiological salt solution (PSS). This 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 arteries were 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 endothelial surface with cotton balls soaked in PSS.

2.3. Measurement of isometric contraction

For the mechanical experiments, the prepared strips were suspended vertically in 5 ml organ baths. One end of each strip was attached to a stainless steel rod, and the other was attached to a force transducer (FT03; Grass-Telefactor). Changes in isometric force were recorded on Grass 79E polygraphs. The strips were suspended under 10 mN resting tension. After equilibration for 20 min in an organ bath filled with PSS, the strips were sequentially exposed to 70 mM KCl and PSS three times. The high KCl solution was prepared by replacing NaCl with equimolar KCl. All bath solutions were thermostatically controlled at 37 °C and were continuously saturated with a mix of 95% O_2 and 5% CO_2 to achieve a pH of 7.4.

2.4. Measurement of MAPK activity

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 $14{,}000 \times g$ for 10 min at 4 °C, and the supernatant was collected. The protein concentrations were determined using Bio-Rad DC protein assay reagents (Bio-Rad, Hercules, CA, USA), 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.

An equivalent amount of aortic protein from both rats, 30–50 μg/lane, was mounted on 10% SDS-PAGE gels. After the separated proteins had been transferred to a polyvinylidene fluoride membrane (Millipore, USA), they were blocked for 1 h with phosphate-buffered saline (PBS) containing 5% non-fat dried milk to prevent non-specific

binding. After blocking, the membranes were washed three times with PBS and 0.05% Tween-20 and then the membranes were incubated with a phosphorylated ERK1/2 and nonphosphorylated ERK1/2 antibodies diluted 1:1000–5000 overnight at 4 °C. The membranes were then incubated with a goat anti-rabbit horseradish peroxidase-conjugated antibody (Amersham, UK) diluted 1:1000 for 1 h at room temperature. Immunoreactivity was detected using enhanced chemiluminescence kits (Amersham). Band intensity was measured by computer analysis, using Quantitation software (Bio-Rad).

2.5. Chemicals

PD098059 (2′-amino-3′-methoxyflavone), GF109203X (2-[1-(3-dimethylaminopropyl)-1*H*-indol-3-yl]-3-(1*H*-indol-3-yl)maleimide), and Y27632 ((*R*)-(+)-*trans*-*N*-(4-Pyridyl)-4-(1-aminoethyl)cyclohexanecarboxamide) were purchased from Tocris (UK). Polyclonal anti-phosphorylated and -nonphosphorylated ERK1/2 antibodies were purchased from Promega (USA). Verapamil, 5-hydroxytryptamine (5-HT), ammonium persulfate, aprotinin, and DOCA were purchased from Sigma (St. Louis, MO, USA). Glycine and Tween 20 were purchased from Bio-Rad. Bromophenol blue, Triton X-100, and low molecular weight electrophoresis calibration kit were purchased from Pharmacia, USA. Ketamine and xylazine were purchased from Yuhan (Korea), and Bayer (Korea), respectively.

2.6. Data analysis

Data are expressed as means±S.E.M. Unpaired Student's *t*-tests were used to compare the data, and $P<0.05$ was considered significantly different.

3. Results

3.1. Blood pressure

Four weeks after the silicon rubber implantation, the mean systolic blood pressure was significantly higher in DOCA-salt hypertensive rats (178±9 mm Hg, $n=14$) than in sham-operated rats (120±6 mm Hg, $n=15$).

3.2. Effects of MAPK inhibitors on myogenic basal tone

To determine whether MAPK influences the basal tone of aortic smooth muscle, we examined the effects of PD098059, an inhibitor of MAPK/ERK kinase, in aortic smooth muscle from sham-operated and DOCA-salt hypertensive rats. In DOCA-salt hypertensive rats, PD098059 (range 10 nM to 10 μM) significantly inhibited myogenic basal tone in a dose-dependent manner (Fig. 1B and C). The inhibitory effects of PD098059 were not recovered by washing with PSS solution for 30 min. In contrast, the aortic strips from sham-operated rats displayed minimal relaxation (<1% of 70 mM K^+-induced contraction, $n=6$) in response to the treatment with PD098059 (Fig. 1A). High K^+-induced contraction was greater in tissues from DOCA-salt hypertensive rats (9.4±0.71 mN per strip, $n=10$) than in sham-operated rats (7.2±0.59 mN per strip, $n=10$). Thus, the basal tone of aortic smooth muscles was significantly greater in the DOCA-salt hypertensive rats than the sham-operated rats, and this elevation of basal tone may be mediated by the MAPK pathway.

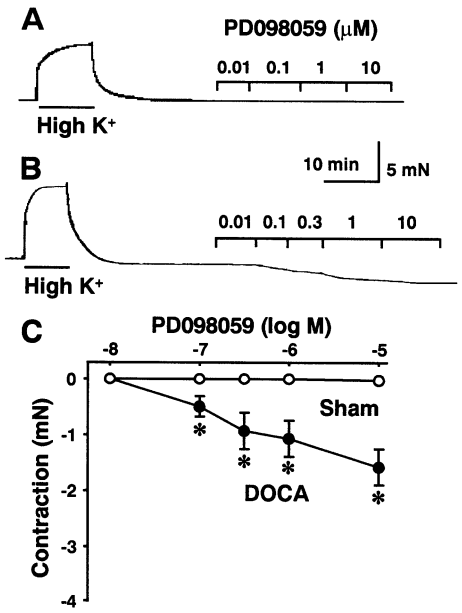

Fig. 1. Effects of PD098059 on basal tone in aortic smooth muscle strips from sham-operated and DOCA-salt hypertensive rats. The muscle strips from sham-operated rats (A) and DOCA-salt hypertensive rats (B) were stimulated repeatedly with 70 mM K^+. After the response to high K^+ had been determined, PD098059 (10 nM to 10 μM) was applied cumulatively in the quiescent state. (C) Dose-response curves of the changes in basal tone induced by PD098059 ($n=4–6$). The contractile values being expressed as mN per muscle strip. *Statistically significantly different from the results of sham-operated control rats ($P<0.05$).

3.3. Effects of MAPK inhibitors on basal MAPK phosphorylation

To confirm the roles of MAPK on basal tone, the activity of MAPK in basal states was measured using a phosphorylated antibody to ERK in aortic smooth muscles from sham-operated and DOCA-salt hypertensive rats. In the quiescent state without any stimulant, phosphorylation of ERK1/2 was significantly greater in DOCA-salt hypertensive rats: 164±13.6% that of the sham-operated rats ($n=6$; Fig. 2B). Treatment with PD098059 at 1 and 10 μM for 15 min decreased basal phosphorylation of ERK1/2 in DOCA-salt hypertensive rats (68.6±9.9%, $n=3$, and 63.8±8.7%, $n=6$, respectively, of the basal level in DOCA). In contrast, the inhibitory effect of PD098059 at 10 μM was not observed in muscle strips from sham-operated rats (115.2±10.5% of the

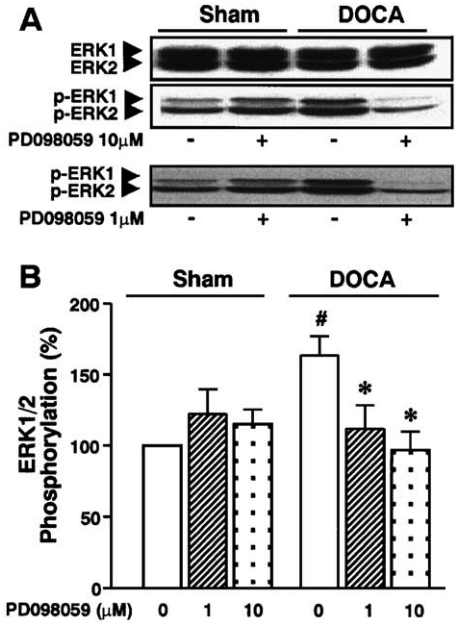

Fig. 2. Effects of PD098059 on ERK1/2 phosphorylation in the aortic smooth muscle strips of sham-operated rats and DOCA-salt hypertensive rats. The strips were prepared as described in Section 2. (A) ERK1/2 phosphorylation in the quiescent and PD098059-treated states was demonstrated using an anti-phosphorylated ERK1/2 antibody on muscle strips. Total ERK1/2 expression was measured using an anti-ERK1/2 antibody on muscle strips from both treatment groups (upper panel). (B) The level of ERK phosphorylation was quantified with the relative density of ERK1/2. The level of phosphorylated ERK1/2 in the quiescent state of sham-operated rats was defined as 100%. Statistical determination of phosphorylated ERK1/2 in both strips was made from 3 to 6 independent experiments. Statistically significantly different from the basal phosphorylation of sham-operated rats (#) and DOCA-salt hypertensive rats (*), respectively ($P<0.05$).

basal levels in controls, $n=6$; Fig. 2). In the western blot analysis using a nonphosphorylated ERK1/2 antibody, the total expression of the kinase in aortic strips in the quiescent state was not changed between sham-operated and DOCA-salt hypertensive rats (Fig. 2A).

3.4. Effects of kinase inhibitors on basal smooth muscle tone and MAPK phosphorylation

To delineate upstream signals that regulate MAPK-dependent basal vascular tone in hypertensive rats, the effects of inhibition of kinases, especially PKC and Rho kinase, were examined. The PKC inhibitor GF109203X, at 10 μM and 30 μM, inhibited basal tone to -1.1 ± 0.41 mN ($n=4$) and -1.2 ± 0.46 mN ($n=4$), respectively, in DOCA-salt hypertensive rats (Fig. 3A). GF109203X at 10 μM and 30 μM induced a minimal inhibition of basal tone in sham-operated rats (-0.04 ± 0.04 mN and -0.1 ± 0.08 mN, respectively, $n=4$). In addition, the Rho kinase inhibitor Y27632, at 1 μM and 10 μM, inhibited basal tone to -1.6 ± 0.54 mN ($n=4$) and -2.3 ± 0.73 mN ($n=4$), respectively, in DOCA-salt hypertensive rats (Fig. 3B). Y27632 at 1 μM and 10 μM slightly inhibited basal tone in the sham-operated rats (-0.2 ± 0.09 mN and -0.34 ± 0.03, respectively, $n=4$). These results suggest that basal vascular tone in the hypertensive rats may be mediated by PKC and Rho/Rho kinase, and by the MAPK pathways. Therefore, to determine whether these kinases influences MAPK-mediated basal tone, the effects of inhibitors on the activity of MAPK was measured in the aortic smooth muscles of sham-operated rats and DOCA-salt hypertensive rats.

GF109203X (10 μM; 20 min) significantly inhibited the basal phosphorylation of ERK1/2 in DOCA-salt hypertensive rats (73.4±4.5% of the basal level in DOCA, $n=7$; Fig. 4). By contrast, this was not observed in muscle strips from sham-operated rats. Y27632 (10 μM; 20 min) failed to inhibit the basal phosphorylation of ERK1/2 in both sham-operated and DOCA-salt hypertensive rats. The total expression of ERK1/2 was not altered by the treatment with GF109203X and Y27632 in sham-operated rats and DOCA-salt hypertensive rats (data not shown). These results suggest that ERK1/2-mediated basal tone is regulated by PKC, but not Rho/Rho kinase, in DOCA-salt hypertensive rats.

3.5. Effects of verapamil on basal tone and MAPK phosphorylation

Vascular basal tone is regulated by $[Ca^{2+}]_i$. To evaluate the contribution of $[Ca^{2+}]_i$ in the regulation of MAPK-mediated basal tone, the effects of Ca^{2+} channel inhibition on basal tone and MAPK activity were measured in aortic trips. Verapamil, an inhibitor of the voltage-dependent Ca^{2+} channel, at 10 μM and 30 μM, decreased basal tone in

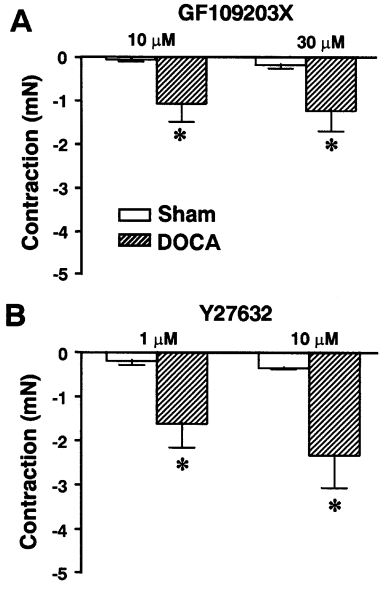

Fig. 3. Effects of kinase inhibitors on basal tone in aortic smooth muscles of sham-operated rats and DOCA-salt hypertensive rats. After the response to high K^+ had been determined, GF109203X (A) and Y27632 (B) were applied to strips in the quiescent state. The contractile values being expressed as mN per muscle strip ($n=4$). * Statistically significantly different from results of sham-operated rats ($P<0.05$).

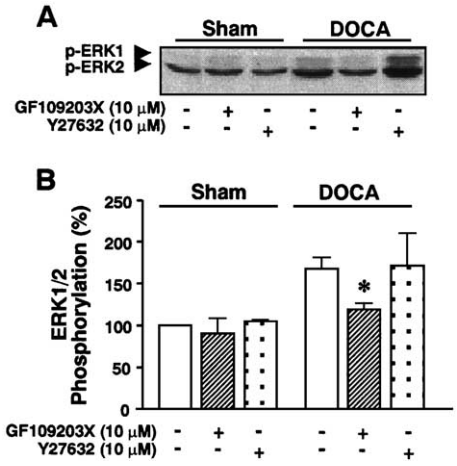

Fig. 4. Effects of kinase inhibitors on basal phosphorylation of MAPK in aortic smooth muscle strips of sham-operated rats and DOCA-salt hypertensive rats. After the response to high K^+ had been determined, GF109203X (A) and Y27632 (B) were applied for 20 min in the quiescent state. The phosphorylation of ERK1/2 was detected using an anti-phosphorylated MAPK antibody, as described in Fig. 2. The basal level of phosphorylated ERK1/2 in sham-operated rats was defined as 100%. Each result represents the mean±S.E.M. of 4–7 experiments. *Statistically significantly different from basal phosphorylation in DOCA-salt hypertensive rats ($P<0.05$).

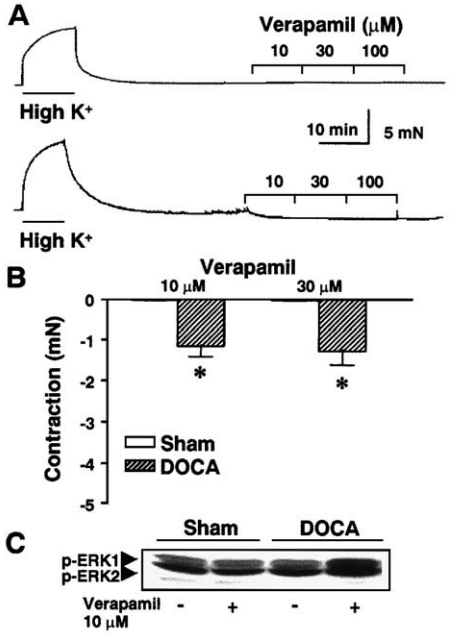

Fig. 5. Effects of verapamil on basal tone and ERK phosphorylation in aortic smooth muscle from sham-operated rats and DOCA-salt hypertensive rats. (A) The muscle strips from sham-operated rats (upper panel) and DOCA-salt hypertensive rats (lower panel) were stimulated repeatedly with 70 mM K^+. After the response to high K^+ had been determined, verapamil was applied in the quiescent state. (B) Statistical analysis of inhibitory effects of verapamil on basal tone ($n=5$). The contractile values being expressed as mN per muscle strip. *Statistically significantly different from results from sham-operated rats ($P<0.05$). (C) Effects of verapamil (10 μM; 20 min) on basal phosphorylation of ERK1/2 in aortic smooth muscle strips of sham-operated rats and DOCA-salt hypertensive rats. Results are representative of three independent experiments.

DOCA-salt hypertensive rats (−1.1±0.25 mN, $n=5$, and −1.3±0.33 mN, $n=5$, respectively, Fig. 5B). In contrast, verapamil did not show an inhibition of the basal tone in muscle strips from sham-operated rats (Fig. 5A and B). In the ERK1/2 phosphorylation measurement experiments, verapamil at 10 μM for 20 min failed to alter the basal phosphorylation of ERK1/2 in both strips (Fig. 5C).

3.6. Roles of MAPK on 5-HT-mediated contraction

Treatment with 5-HT (1 nM to 100 μM) induced sustained contractions in dose-dependent manners in both sham-operated and DOCA-salt hypertensive rats (Fig. 6A and B). The response to 5-HT was greater in the experimental rats than in sham-operated controls. 5-HT (10 μM) increased the phosphorylation of ERK1/2 in both strips, and these increases in ERK1/2 phosphorylation were greater in strips from DOCA-salt hypertensive rats than in sham-operated controls (Fig. 6C), as in our previous report (Kim et al., 2004).

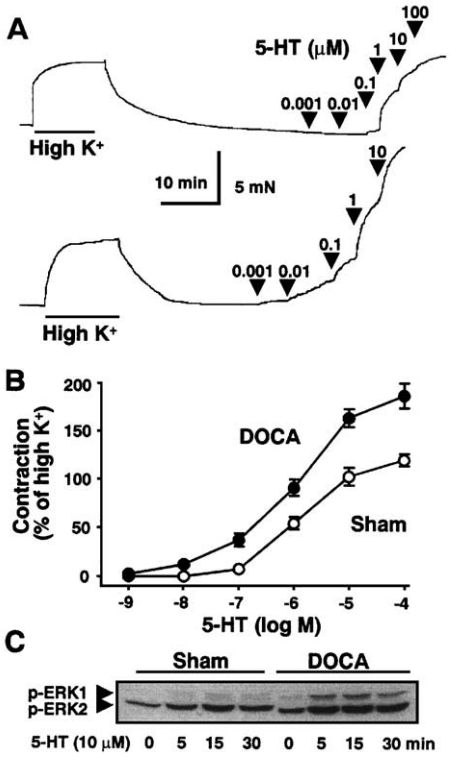

Fig. 6. Effects of 5-hydroxytryptamine on contraction and ERK1/2 phosphorylation in aortic smooth muscle strips from sham-operated rats and DOCA-hypertensive rats. (A) The muscle strips from sham-operated (upper panel) and DOCA-hypertensive (lower panel) rats were stimulated repeatedly with 70 mM K^+. After the response to high K^+ had been determined, 5-HT (1 nM to 100 μM) was applied cumulatively. (B) Dose–response curves of the changes in contraction induced by 5-HT. The contractile level in response to 70 mM K^+ before treatment with 5-HT was defined as 100% ($n=3$). (C) 5-HT (10 μM)-induced elevation of ERK1/2 phosphorylation in aortic smooth muscle. These results are representative of three independent experiments.

4. Discussion

We found here that MAPK inhibitors significantly inhibited the basal tone in vascular smooth muscle from DOCA-salt hypertensive rats. Moreover, the inhibitor attenuated the basal activity of ERK1/2 in the hypertensive rats. By contrast, the inhibitor did not alter either the basal tone or basal activity of MAPK in sham-operated normotensive rats. These closely matched results between mechanical and MAPK activities strongly suggest that the MAPK pathway is involved in the regulation of basal tone in vascular smooth muscle from hypertensive rats, and that this mechanism is significantly greater in DOCA-salt hypertensive rats than in sham-operated normotensive rats. In the present study, we also found that GF109203X the PKC inhibitor, at a concentration previously used for the inhibition of PKC (Lee and Shukla, 2004; Jackson et al., 2004), inhibited both the basal tone and MAPK activity in the hypertensive rats but not in sham-operated rats. Thus, PKC is essential for MAPK-mediated basal tone in hypertensive rats. In addition, it has been reported that the Ca^{2+}-dependent and -independent isoform of PKC can activate MAPK in vascular smooth muscle (Liao et al., 1997). Thus, PKC can be upstream to the MAPK pathways that regulate the increment of basal tone in hypertensive rats.

PI3K activity is increased in hypertensive rats and this kinase may be involved in enhanced contractility (Northcott et al., 2004). Moreover, inhibition of PI3K attenuated the basal tone in DOCA-salt hypertensive rats, implying that PI3K may also be involved in the regulation of basal tone. Although PI3K activity is increased in hypertensive rats, Akt, downstream to PI3K, is decreased, suggesting that there must be an unknown effector regulating basal tone in hypertensive rats (Northcott et al., 2002). It has been reported that PI3K is upstream to MAPK in the smooth muscle tone control pathway (Campbell et al., 2004; Yart et al., 2001). We therefore suggest that MAPK is a plausible candidate for the factor downstream to PI3K in elevating basal vascular tone in hypertensive rats.

The present results showed that Y27632 the Rho kinase inhibitor strongly inhibited the basal tone in DOCA-salt hypertensive rats. By contrast, Y27632 did not produce any change in the MAPK pathway, although muscle strips were treated with a higher concentration of the inhibitor. The activation of Rho in basal state is increased in hypertensive rats (Seko et al., 2003). Thus the Rho pathway contributes to the regulation of basal tone in hypertensive rats, but this pathway may be dissociated from the MAPK-mediated basal tone. This result is supported by the previous report that basal tone is regulated by both the PI3K and the Rho/Rho kinase pathways, and that these pathways act in parallel in supporting arterial tone without an interaction between two signals (Wehrwein et al., 2004).

In addition to the MAPK pathway, the Ca^{2+} channel inhibitor verapamil strongly decreased the basal tone in the hypertensive rats but not in normotensive rats. It is well known that exposure to Ca^{2+} free media and to inhibitors of voltage-dependent Ca^{2+} channels decrease basal tone in spontaneously hypertensive rats (Zou et al., 1995). Thus the basal tone obtained in the hypertensive rats depends on Ca^{2+} influx through channels in the smooth muscle cell plasma membrane. However, we found, in the present study, treatment of the muscle strips with verapamil did not alter the MAPK activity in hypertensive rats. Moreover, Ca^{2+} removal failed to decrease the activity of MAPK increased by vasoconstrictor in aortic smooth muscle (Park et al., 2003). These results indicate that the basal vascular tone can be mediated by $[Ca^{2+}]_i$, as well as the MAPK pathway, and that there is no contribution of $[Ca^{2+}]_i$ in MAPK-mediated basal tone in the hypertensive rats. The Rho/Rho kinase-mediated basal tone can be regulated by $[Ca^{2+}]_i$ (Nakamura et al., 2003; Wehrwein et al., 2004). It can therefore be assumed that there are multiple pathways to regulate basal tone in vascular smooth muscle strips from hypertensive rats, including the Ca^{2+}-dependent Rho/Rho kinase pathway and the PI3K- and/or PKC-mediated MAPK pathway.

In the present study, the levels of MAPK in the quiescent state were significantly increased in the muscles of DOCA-salt hypertensive rats, which is consistent with previous reports on aortic smooth muscle from spontaneous and DOCA-salt hypertensive rats (Kubo et al., 2002; Touyz et al., 2002). Moreover, vasoconstrictor-induced contraction is mediated by MAPK activation, which consequently results in the increased phosphorylation of caldesmon, an actin-binding protein. The phosphorylation of caldesmon in the quiescent state was significantly increased in strips from the DOCA-salt hypertensive rats, implying that it may be associated with greater basal tone in hypertensive vessels (Kim et al., 2004). Furthermore, in the present study and previous reports, the magnitude of contractility and activity of MAPK induced by vasoconstrictors was greater in DOCA-salt hypertensive rats than in normotensive rats (Tostes et al., 2000). Furthermore, the application of a MAPK inhibitor attenuated systolic blood pressure in hypertensive rats (Muthalif et al., 2000). From these results, it can be assumed that the increases in activity in the MAPK-related pathways in both quiescent and vasoconstrictor-stimulated states may contribute to the elevation of blood pressure in DOCA-salt hypertensive rats.

Here we showed that the basal tone and ERK1/2 activity were significantly higher in DOCA-salt hypertensive rats than in sham-operated rats, and that inhibitor of MAPK inhibited the basal tone and the activity of ERK1/2 in the hypertensive rats. Furthermore, the MAPK-mediated basal tone was regulated by PKC, but not by $[Ca^{2+}]_i$ and/or Rho/Rho kinase in the hypertensive rats. In conclusion, basal vascular tone in DOCA-salt hypertensive rats is elevated by an altered activation of MAPK via PKC.

Acknowledgement

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References

Adam, L.P., Franklin, M.T., Raff, G.J., Hathaway, D.R., 1995. Activation of mitogen-activated protein kinase in porcine carotid arteries. Circ. Res. 76, 183–190.

Campbell, M., Allen, W.E., Sawyer, C., Vanhaesebroeck, B., Trimble, E.R., 2004. Glucose-potentiated chemotaxis in human vascular smooth muscle is dependent on cross-talk between the PI3K and MAPK signaling pathways. Circ. Res. 95, 380–388.

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

Epstein, A.M., Throckmorton, D., Brophy, C.M., 1997. Mitogen-activated protein kinase activation: an alternate signaling pathway for sustained vascular smooth muscle contraction. J. Vasc. Surg. 26, 327–332.

Ghosh, M., Hanna, S.T., Wang, R., McNeill, J.R., 2004. Altered vascular reactivity and K_{ATP} channel currents in vascular smooth muscle cells from deoxycorticosterone acetate (DOCA)-salt hypertensive rats. J. Cardiovasc. Pharmacol. 44, 525–531.

Jackson, T.C., Mi, Z., Jackson, E.K., 2004. Modulation of cyclic AMP production by signal transduction pathways in preglomerular microvessels and microvascular smooth muscle cells. J. Pharmacol. Exp. Ther. 310, 349–358.

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

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

Krum, H., Viskoper, R.J., Lacourciere, Y., Budde, M., Charlon, V., 1998. Bosentan hypertension investigators: the effect of an endothelin-receptor antagonist, bosentan, on blood pressure in patients with essential hypertension. N. Engl. J. Med. 338, 784–790.

Kubo, T., Ibusuki, T., Chiba, S., Kambe, T., Fukumori, R., 2002. Altered mitogen-activated protein kinase activation in vascular smooth muscle cells from spontaneously hypertensive rats. Clin. Exp. Pharmacol. Physiol. 29, 537–543.

Lee, Y.J., Shukla, S.D., 2004. Pro- and anti-apoptotic roles of c-Jun N-terminal kinase (JNK) in ethanol and acetaldehyde exposed rat hepatocytes. Eur. J. Pharmacol. 508, 31–45.

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

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

Nakamura, A., Hayashi, K., Ozawa, Y., Fujiwara, K., Okubo, K., Kanda, T., Wakino, S., Saruta, T., 2003. Vessel- and vasoconstrictor-dependent role of rho/rho-kinase in renal microvascular tone. J. Vasc. Res. 40, 244–251.

Northcott, C.A., Poy, M.N., Najjar, S.M., Watts, S.W., 2002. Phosphoinositide 3-kinase mediates enhanced spontaneous and agonist-induced contraction in aorta of deoxycorticosterone acetate-salt hypertensive rats. Circ. Res. 91, 360–369.

Northcott, C.A., Hayflick, J.S., Watts, S.W., 2004. PI3-kinase upregulation and involvement in spontaneous tone in arteries from DOCA-salt rats: is p110delta the culprit? Hypertension 43, 885–890.

Osol, G., Laher, I., Cipolla, M., 1991. Protein kinase C modulates basal myogenic tone in resistance arteries from the cerebral circulation. Circ. Res. 68, 359–367.

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

Seko, T., Ito, M., Kureishi, Y., Okamoto, R., Moriki, N., Onishi, K., Isaka, N., Hartshorne, D.J., Nakano, T., 2003. Activation of RhoA and inhibition of myosin phosphatase as important components in hypertension in vascular smooth muscle. Circ. Res. 92, 411–418.

Somlyo, A.P., Himpens, B., 1989. Cell calcium and its regulation in smooth muscle. FASEB J. 3, 2266–2276.

Stoclet, J.C., Chataigneau, T., Ndiaye, M., Oak, M.H., El Bedoui, J., Chataigneau, M., Schini-Kerth, V.B., 2004. Vascular protection by dietary polyphenols. Eur. J. Pharmacol. 500, 299–313.

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

Touyz, R.M., Mabrouk, M.E., He, G., Wu, X.H., Schiffrin, E.L., 1999. 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.

Touyz, R.M., Deschepper, C., Park, J.B., He, G., Chen, X., Neves, M.F., Virdis, A., Schiffrin, E.L., 2002. Inhibition of mitogen-activated protein/extracellular signal-regulated kinase improves endothelial function and attenuates Ang II-induced contractility of mesenteric resistance arteries from spontaneously hypertensive rats. J. Hypertens. 20, 1127–1134.

Wehrwein, E.A., Northcott, C.A., Loberg, R.D., Watts, S.W., 2004. Rho/Rho kinase and phosphoinositide 3-kinase are parallel pathways in the development of spontaneous arterial tone in deoxycorticosterone acetate-salt hypertension. J. Pharmacol. Exp. Ther. 309, 1011–1019.

Yart, A., Laffargue, M., Mayeux, P., Chretien, S., Peres, C., Tonks, N., Roche, S., Payrastre, B., Chap, H., Raynal, P., 2001. A critical role for phosphoinositide 3-kinase upstream of Gab1 and SHP2 in the activation of Ras and mitogen-activated protein kinases by epidermal growth factor. J. Biol. Chem. 276, 8856–8864.

Zou, H., Ratz, P.H., Hill, M.A., 1995. Role of myosin phosphorylation and $[Ca^{2+}]_i$ in myogenic reactivity and arteriolar tone. Am. J. Physiol. 269, H1590–H1596.